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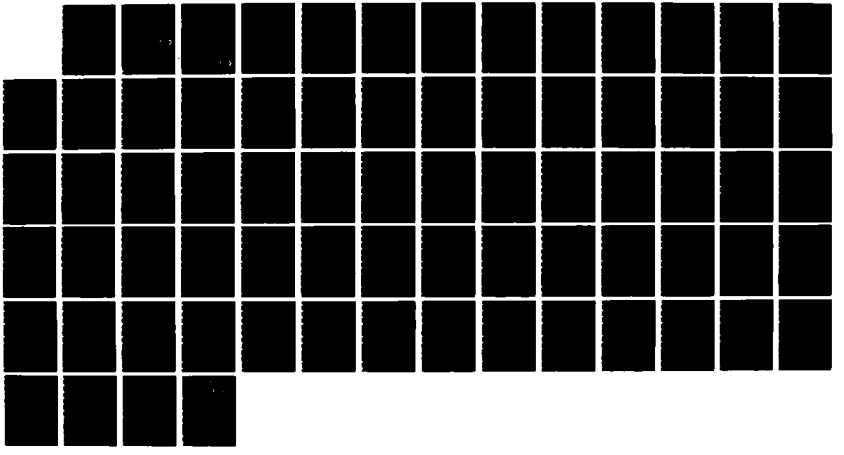

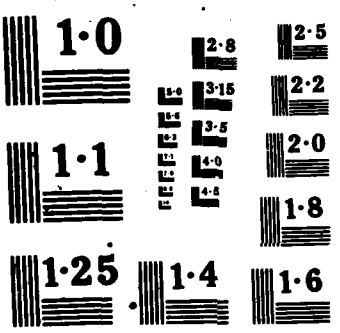

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NEUROTOXICOLOGY OF CYCLOTRIMETHYLENETRINITRAMINE (RDX)

FINAL REPORT

R.C. MacPhail[1], Q.D. Walker[2], and L.L. Cook[1]

JUNE 1985

Supported by:

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21701-5012

Project Order 2813

[1]Neurotoxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27514

[2]Northrop Services, Inc.
Environmental Sciences
Research Triangle Park, NC 27709

Project Officer: Gunda Reddy, Ph.D.
Health Effects Research Division
U.S. ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT LABORATORY
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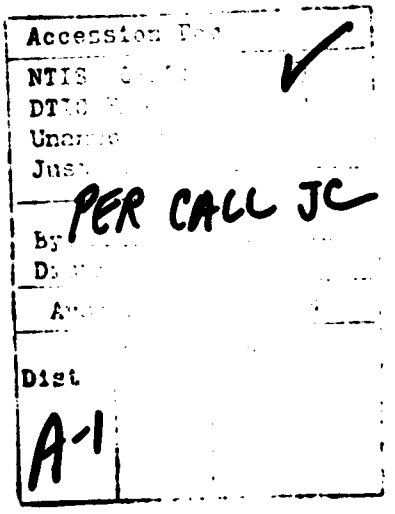

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report summarizes the results of a series of experiments designed to determine in rats the behavioral effects of acute and subchronic exposure to RDX. The behavioral endpoints included measures of sensory (acoustic startle), motor (landing footspread, figure-eight maze activity) and cognitive (flavor-aversion conditioning, acquisition and performance of schedule-controlled behavior) capacity. Since the behavioral evaluations were to be carried out using newly assembled equipment in a newly constructed

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laboratory, several preliminary behavioral experiments were executed to insure that the equipment and test conditions were appropriate. Additional pilot experiments determined the appropriate conditions for measuring RDX levels in blood and brain, and the time course of RDX levels in blood and in brain.

Phase I involved determining the acute effects of RDX. The results of these experiments showed that RDX produced a multitude of behavioral effects. More specifically, when compared to vehicle-treated (2% carboxymethylcellulose in water) rats, RDX (12.5-50 mg/kg), p.o., 2-hr presession produced: (1) dosage-related decreases in startle-response amplitude and dosage-related increases in startle-response latency; (2) dosage-related decreases in figure-eight maze motor activity; (3) decreases (not dosage-related) in landing footspread; (4) dosage-related conditioned flavor aversions; and (5) dosage-related decreases in rates of schedule-controlled responding (VR 50-response and VI 90-second). In most instances these effects were substantial and apparent after even the smallest dosage, which is perhaps about 10% of the LD50. In addition, in many instances carryover effects were noted following acute RDX administration. On the basis of these data, we conclude that acute RDX exposures are likely to be associated with multiple deficits in neurobehavioral integrity.

Results of the analytical chemistry experiments showed that: (1) levels of RDX in plasma and in brain were a direct function of the amount administered; and (2) following a large dosage (50 mg/kg), RDX levels in plasma (3-4 μg/ml) and brain (5-9 μg/g) ordinarily reached a steady state two hours after dosing and remained so for up to 24 hours after dosing. A further experiment showed that RDX levels disappeared by three days after administration of 50 mg/kg.

Phase II involved determining the subchronic effects of RDX. Rats received p.o. either the vehicle or 1, 3, or 10 mg/kg/day of RDX for 30 days. Testing occurred before, during and after subchronic dosing. The results of these experiments showed that, in contrast to its acute effects, subchronic exposures to RDX generally did not result in significant neurobehavioral impairment. The general lack of effect in these experiments may have been due to the particular dosages, the chronicity with which the dosages were administered, and/or the test times selected relative to dosing.

Recommendations for further studies include defining the conditions under which subchronic RDX exposures produce behavioral effects, as well as determining the metabolism and toxicokinetics of RDX under a variety of dosing protocols.

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EXECUTIVE SUMMARY

This report summarizes the results of a series of experiments designed to determine in rats the behavioral effects of acute and subchronic exposure to RDX. The behavioral endpoints included measures of sensory (acoustic startle), motor (landing footspread, figure-eight maze activity) and cognitive (flavor-aversion conditioning, acquisition and performance of schedule-controlled behavior) capacity. Since the behavioral evaluations were to be carried out using newly assembled equipment in a newly constructed laboratory, several preliminary behavioral experiments were executed to insure that the equipment and test conditions were appropriate. Additional pilot experiments determined the appropriate conditions for measuring RDX levels in blood and brain, and the time course of RDX levels in blood and in brain.

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Recommendations for further studies include defining the conditions under which subchronic RDX exposures produce behavioral effects, as well as determining the metabolism and toxicokinetics of RDX under a variety of dosing protocols.

LIST OF FIGURES

I. INTRODUCTION AND RESEARCH PLAN

RDX (cyclonite, cyclotrimethylenetrinitramine, or hexahydro-1,3,5-trinitro-1,3,5-triazine; CAS No. 121-82-4) is a widely used military explosive. Early reports of poisoning episodes indicated that occupationally exposed individuals displayed generalized convulsions of the clonic-tonic type that may have been preceded by irritability, insomnia and restlessness (see 4). Severe nausea was also common, along with deficits in concentration and memory. Most poisoning episodes have involved inadvertent exposures. Since its widespread use in Vietnam, however, it is now known that exposures may also be intentional, as ingestion reportedly produces a "high" similar to that of ethanol (5), thereby adding a new dimension to its clinical toxicity.

Laboratory studies by von Oettingen et al. (14) showed that daily feeding of RDX (50-100 mg/kg) to rats for 10 weeks produced hyperreactivity and convulsions. Affected rats were described as being vicious, biting for example cage mates and also laboratory attendants when being handled. These effects disappeared in surviving rats on discontinuation of daily dosing. Similar effects were not seen in rats fed 15 mg/kg daily. Levine et al. (6) reported that rats receiving large dosages (100-600 mg/kg) of RDX daily via the diet for up to 13 weeks displayed hyperreactivity to approach. In addition, tremors and convulsions were reported in some of the rats receiving the largest dosage daily. Histopathological analysis of the nervous system revealed no grossly observable lesions. Schneider et al. (13), on the other hand, reported no neurological signs in rats that received daily intubations of a small dosage of RDX (20 mg/kg) for up to 90 days.

The findings of seizures and hyperreactivity in rats treated subchronically with RDX, together with the clinical reports of RDX exposure, suggest that RDX has neurotoxic properties. The present series of experiments was therefore undertaken to determine the behavioral effects of acute and subchronic administration of RDX to rats. In contrast to previous work, the present experiments used precisely specified behavioral tests to evaluate the effects of RDX exposures. The tests selected were: acoustic startle response, flavor-aversion conditioning, schedule-controlled performance, figure-eight maze motor activity and landing footspread. The acoustic startle response and landing footspread are reactions to events taking place in the environment. Motor activity measured in the figure-eight maze is "spontaneous" in the sense that no prior training of the animals is required for its occurrence. Conditioned flavor aversions and schedule-controlled behavior, on the other hand, are derived responses that are acquired on the basis of experience. In this way we attempted to broadly evaluate the sensory, motor and cognitive effects of RDX exposures. In addition, RDX levels in plasma and in whole brain were determined in order to estimate concentrations likely to produce behavioral effects.

II. METHODS

A. Animals. All experiments were carried out using adult Sprague-Dawley-derived (CD) rats received from Charles River Breeding Co. (Wilmington, MA). Preliminary experiments used primarily female rats whereas the acute and the subchronic experiments used primarily male rats. One series of acute experiments (schedule-controlled behavior) used both male and female rats and therefore permitted a direct determination of the extent to which the behavioral effects of RDX depended on the sex of the animals. Table 1 provides a summary of the animals used in each of the experiments.

B. Behavioral

1. Motor activity. Motor activity was measured in figure-eight mazes during one-hour sessions. The figure-eight maze has been described in detail by Reiter (10) and consists of a series of interconnecting alleys (10 cm x 10 cm) shaped like a figure-eight that converge with two blind alleys on a central open arena and is covered with transparent acrylic plastic. Eight pairs of photoemitters and detectors are spaced strategically around the figure-eight maze to record activity (three in each half of the figure-eight portion and one in each of the blind alleys). In the acute experiment, testing took place during single 60-min sessions beginning two hours after dosing and again on the next day. During the subchronic experiment, testing took place during 60-min sessions on the day before dosing began, and on days 16 and 31 of the subchronic regimen. Data collected included the total number of photobeam interruptions, the relative within-session temporal distribution of motor activity (analyzed separately in 5 successive 12-min blocks), and the spatial distribution of motor activity (i.e., the relative number of photocell interruptions occurring in the blind alleys).

Table 1. Summary of the animals used in each experiment, including special conditions.

Experiment	Toxicant	N	Sex	Age at testing (days)	Special conditions
1. Motor activity	d-Amphetamine (acute)	32	F	83	None
2. Motor activity	Chlorpromazine (acute)	32	F	82	None
3. Flavor aversion	Lithium chloride (acute)	36	F	128	Water-deprived
4. Flavor aversion	Lithium chloride (acute)	8	M	96	Water-deprived
5. Schedule-controlled behavior	RDX (acute)	24	F	136	Food-deprived
6. Motor activity/ landing footspread	RDX (acute)	32	M	60	None
7. Flavor aversion	RDX (acute)	36	M	66	Water-deprived
8. Schedule-controlled behavior	RDX (acute)	64	M	94	Food-deprived
9. Schedule-controlled behavior	RDX (acute)	64	F	110	Food-deprived
10. Acoustic startle	RDX (acute)	40	M	68	None

Table 1 (continued). Summary of the animals used in each experiment, including special conditions.

	Experiment	Toxicant	N	Sex	Age at testing (days)	Special conditions
11.	Motor activity/ landing footspread	RDX (subchronic)	32	M	65	None
12.	Flavor aversion	RDX (subchronic) & Lithium chloride (acute)	72	M	64	Water-deprived
13.	Schedule-controlled behavior	RDX (subchronic)	32	M	90	Food-deprived
14.	Acoustic startle	RDX (subchronic)	40	M	61	None
15.	Acoustic startle	RDX (subchronic)	40	M	76	None

2. Landing footspread. Landing footspread was carried out according to the method of Edwards and Parker (2). Briefly, the hindpaws of each rat were first inked. The rat was then held elevated in a horizontal position, approximately 30 cm above a table covered with white paper, and dropped twice. The distance between the midpoint of the hindpaws was then determined and averaged for each rat across the two trials. Landing footspread was determined in the same rats used to measure motor activity, and in all instances this was done shortly after the motor activity test session.

3. Flavor-aversion conditioning. Flavor-aversion conditioning was carried out in a manner similar to that described by MacPhail (8). Briefly, all rats received restricted daily access (30 min/day) to deionized water in their home cages. Food was always freely available. Once intakes had stabilized (ordinarily within three weeks), saccharin solution (0.1% w/v) was substituted for water for one 30-min session. Approximately 20 min after saccharin availability had terminated, rats were treated with a dosage of the toxicant of interest, the toxicant vehicle or nothing. Three days after dosing all rats were given a 30-min choice between consuming saccharin and deionized water. Lithium chloride was used as the reference (positive control) toxicant in the preliminary experiment and in the subchronic experiment, whereas RDX was used in the acute experiment. Choice in the acute experiment was determined one day as well as three days after dosing with RDX. In the subchronic experiment, saccharin-lithium pairings were arranged on day 31 of the regimen (i.e., one day after the 30th daily treatment with RDX or RDX vehicle). In all experiments relative saccharin intake (expressed as a proportion of total intake) and total intake were determined.

4. Schedule-controlled behavior. Tests of schedule-controlled behavior were carried out in the following way. Eight commercial operant conditioning chambers were used (Model E10-10, Coulbourn Instruments, Inc.). Each chamber was equipped with a circular response key (Coulbourn Model E21-17), a dipper device capable of delivering liquids (0.25 ml) to the chamber, a house light and a series of cue lights. Each chamber was housed in a larger sound- and light-attenuating chamber. Each rat was trained to respond (press the key) using access (4 sec) to a solution of one part Borden's Eagle Brand sweetened condensed milk: two parts tap water as the reinforcer. Sessions were signalled by illumination of the houselight whereas reinforcement was signalled by termination of the houselight and illumination of a light located above the dipper. Performances were maintained under either a variable-interval 90-sec (VI 90-sec) or a variable-ratio 50-response (VR-50) schedule. Under the VI 90-sec schedule, a response intermittently produced access to milk; the intervals separating successive milk availability periods were generated from a mathematical formula (constant probability) reported by Fleshler and Hoffman (3) and averaged 90 seconds in length. Under the VR-50 schedule a response intermittently produced access to milk depending on the number of responses that had preceded it; successive response requirements were taken from the formula of Fleshler and Hoffman (3) and averaged 50 responses. Sessions were approximately one-half hour in length and were terminated by either: (a) 81 reinforcements; (b) the first reinforcement after 30 minutes; or (c) 33 minutes of session time (whichever occurred first). Data collected included the total number of responses and reinforcements per session.

5. Acoustic startle response. Acoustic startle response was determined in chambers described by Ruppert et al. (11). Briefly each

rat was placed in a small chamber that rested on a force transducer and was enclosed in a larger sound- and light-attenuating chamber equipped with two speakers. A microprocessor automatically controlled delivery of the startle stimulus and recording of the data. The startle stimulus consisted of a 13-kHz 120-dB tone presented for 40 msec. Each session consisted of ten presentations of the startle stimulus in the presence of each of three background white noise intensities (45, 60, 75 dB). Latency and amplitude of the startle response were recorded at each background intensity for each rat.

B. Analytical. In all experiments rats were killed by decapitation and trunk blood was collected in 4-ml Vacutainer (Becton-Dickinson) test tubes containing 6 mg of EDTA as an anticoagulant. The tubes were gently inverted to mix the blood and were immediately placed on ice until centrifugation. Plasma was removed from blood samples by centrifugation at 1,000 x G for 15 min and stored frozen in polypropylene test tubes until analysis. Whole brains were removed immediately after decapitation and stored frozen in glass scintillation vials until analysis.

Plasma and brain concentrations of RDX were determined by extraction into water-washed ethyl acetate followed by analysis using high pressure liquid chromatography (HPLC). Plasma (0.5 ml) was transferred to 5-ml polypropylene test tubes (Walter Sarstedt) followed by the addition of benzophenone (Sigma Chemical Co.) as an internal standard. External standards were prepared by adding known amounts of RDX to plasma from vehicle-treated rats. The tubes were capped and mixed horizontally on a shaker for 10-15 min followed by centrifugation at 10,000 x G for 15 min to separate the aqueous and organic phases. Aliquots of ethyl-acetate phase of all samples were transferred to 0.25-ml polypropylene microcentrifuge tubes which were placed in sealed sample vials for automatic injection into the HPLC.

Whole brains were homogenized by a motorized glass/Teflon homogenizer in 4.0 ml of deionized water. One ml of the homogenate was extracted into 0.5 ml of ethyl acetate as described above. Additional details, including determination of the efficiency and sensitivity of extraction into ethyl acetate are provided in Appendix.[1]

The HPLC equipment used in the analysis consisted of an automatic injector, pump, C-18 reverse phase column and a UV detector (Waters Associates). The mobile phase was 65% methanol (Burdick and Jackson) and 1.82 mM heptane sulfonic acid (Fisher Scientific Co.), and absorption was monitored at 254 nm.

A preliminary experiment was carried out to determine RDX levels in plasma and brain at varying times (1-24 hr) following p.o. administration of 50 mg/kg RDX. An additional time course experiment determined RDX levels in plasma and brain 1-4 days after dosing with 50 mg/kg. In the acute experiment, RDX levels were determined approximately three hours after dosing with 0 (i.e., vehicle), 12.5, 25 or 50 mg/kg RDX. In the subchronic experiment, RDX levels were determined in rats killed on day 16 or day 31 of the dosing regimen.

C. Dosing.

1. Drugs. d-Amphetamine SO_4, chlorpromazine HCl and lithium chloride (Sigma Chemical Co., Chicago, IL) were dissolved in isotonic saline solution and administered i.p. in a volume of 1 ml/kg b. wt. Dosages of d-amphetamine and chlorpromazine are expressed as mg of the total salt/kg b. wt. Dosages of lithium chloride are expressed as mEq/kg b. wt. (1 mEq = 42.3 mg/kg).

2. RDX. RDX (cyclotrimethylenetrinitramine; CAS No. 121-82-4) was obtained from a 5-lb batch retained by IIT Research Institute (Chicago, IL) and identified as Lot HOL 435-37. The RDX used in these experiments was taken from the same lot used in (6), which had a stated purity of 84.7 $\pm$4.7%. A 50-g sample was then ball-milled by Dr. R. Remaly (IITRI).

Particle-size analysis indicated that approximately 90% of the particles were smaller than or equal to 66 μm in diameter. The distribution of particle sizes agreed very well with that used in (6). RDX was suspended in a 2% solution of carboxymethylcellulose and administered p.o. in a volume of 1 ml/kg b. wt. RDX was prepared shortly before acute dosing and at 2-3 day intervals during subchronic dosing. In the acute toxicity experiments, RDX was administered in dosages of 0 (vehicle only), 12.5, 25 and 50 mg/kg b. wt. Testing ordinarily occurred two hours after dosing. In the subchronic toxicity experiments, RDX was administered for 30 days in dosages of 0 (vehicle only), 1, 3 and 10 mg/kg b. wt./day. Testing ordinarily occurred before, during and after subchronic dosing. Testing during subchronic dosing occurred on the day after a daily treatment but prior to that day's treatment.

III. RESULTS

A. Preliminary experiments. Several preliminary experiments were carried out to insure that the test conditions and equipment were appropriate before work with RDX was undertaken. These studies involved determining the effects of reference drugs (d-amphetamine and chlorpromazine) on motor activity measured in figure-eight mazes, flavor-aversion conditioning using a prototype drug (lithium chloride), as well as establishing baselines of performance under a variable-interval 90-second (VI 90-sec) or a variable-ratio 50-response (VR-50) schedule of milk delivery and determining the effect of a single dosage of RDX or RDX vehicle on performance.

1. Motor activity. Separate groups of female rats were treated with either a dosage of d-amphetamine, chlorpromazine or the isotonic saline vehicle. Motor activity was then evaluated during a single 60-min

session in figure-eight mazes. The results of these experiments are shown in Figure 1. d-Amphetamine produced increases in motor activity that were maximal at 1.0 mg/kg. A larger dosage generally decreased motor activity, although the substantial between-subject variability seen in Figure 1 indicates that this dosage produced both activity increases and activity decreases in different rats. Chlorpromazine, on the other hand, generally decreased responding in a dosage-related fashion (Figure 1).

The effects of d-amphetamine and chlorpromazine on the within-session distribution of motor activity are shown in Figure 2. Vehicle-treated control rats displayed a decay of motor activity within the session that appeared to reach asymptotic levels late in the session. d-Amphetamine produced biphasic effects on the within-session distribution of motor activity. The smaller dosages attenuated the within-session decay of motor activity whereas the largest dosage accentuated the decay. The smaller dosages of chlorpromazine, on the other hand, were generally without effect. The largest dosage of chlorpromazine accentuated the within-session decay of motor activity, although it did not affect the asymptote of activity obtained late in the session.

The effects of d-amphetamine and chlorpromazine on the spatial distribution of motor activity in the figure-eight maze are shown in Figure 3. Rats receiving saline were just slightly less active in the blind alleys of the figure-eight maze than would be predicted on the basis of chance. d-Amphetamine decreased the relative number of photocell interruptions in the blind alleys, but only at the largest dosage, whereas chlorpromazine was without effect at all dosages.

2. Flavor-aversion conditioning. Female rats received daily restricted access (30 min/day) to water. Once intakes had stabilized, a saccharin solution was substituted for water, and approximately 20 min

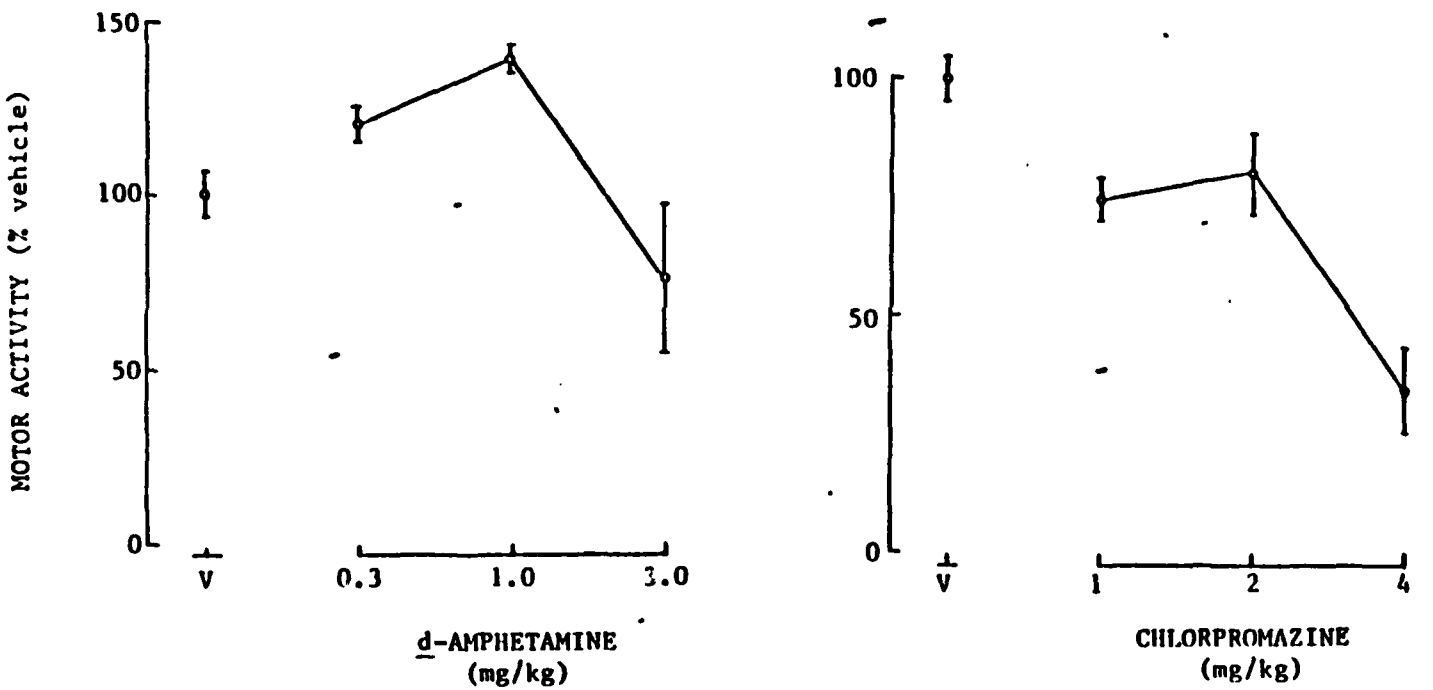

Figure 1. Drug effects on motor activity. Each symbol represents the mean (±SEM) effect of d-amphetamine or the saline vehicle (left panel), and chlorpromazine or the saline vehicle (right panel), on the figure-eight maze activity of female rats (N=8/group).

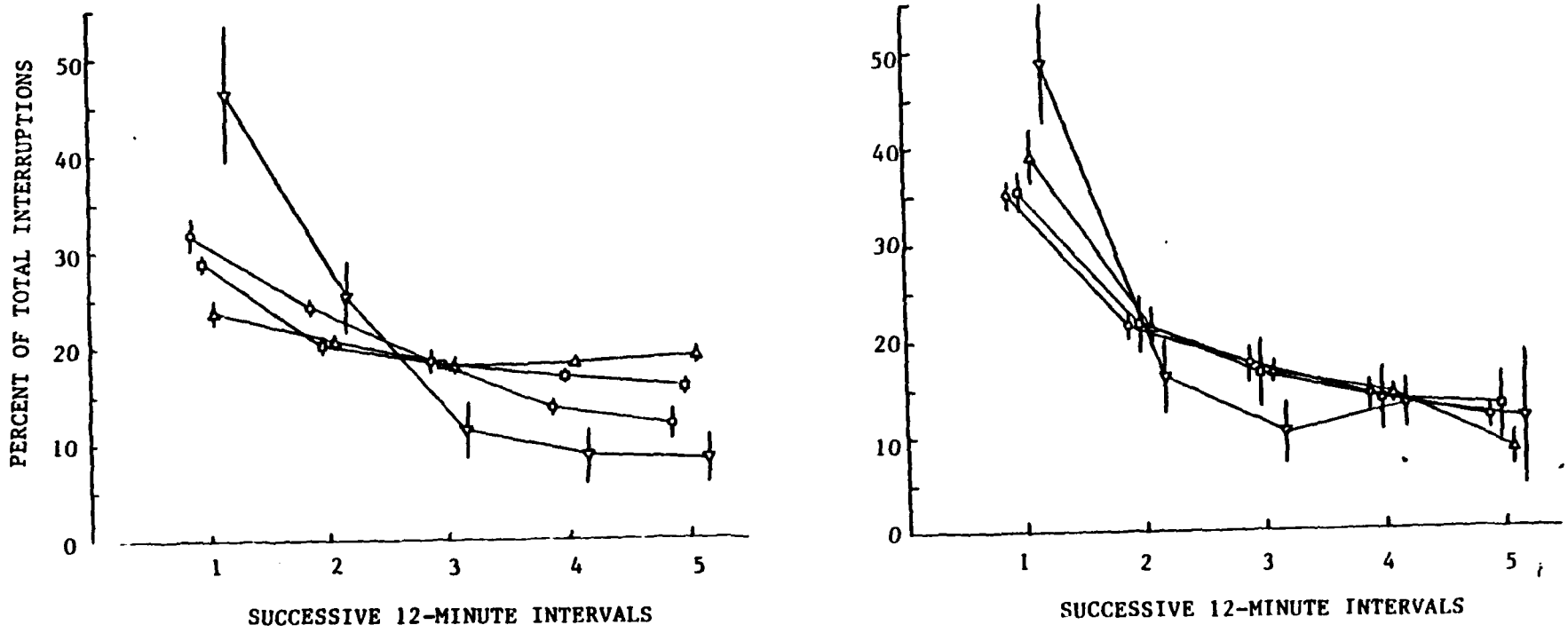

Figure 2. Drug effects on the within-session distribution of motor activity. Each symbol represents the mean (±SEM) effect of d-amphetamine (squares, 0.3 mg/kg; triangles, 1.0 mg/kg; inverted triangles, 3.0 mg/kg) or the saline vehicle (circles) (left panel), and chlorpromazine (squares, 1 mg/kg; triangles, 2 mg/kg, inverted triangle 4 mg/kg) or the saline vehicle (circles) (right panel), on the motor activity of female rats, expressed as a percentage of total activity, across successive 12-min periods of a one-hour test session. Symbols have been displaced for ease of visual representation.

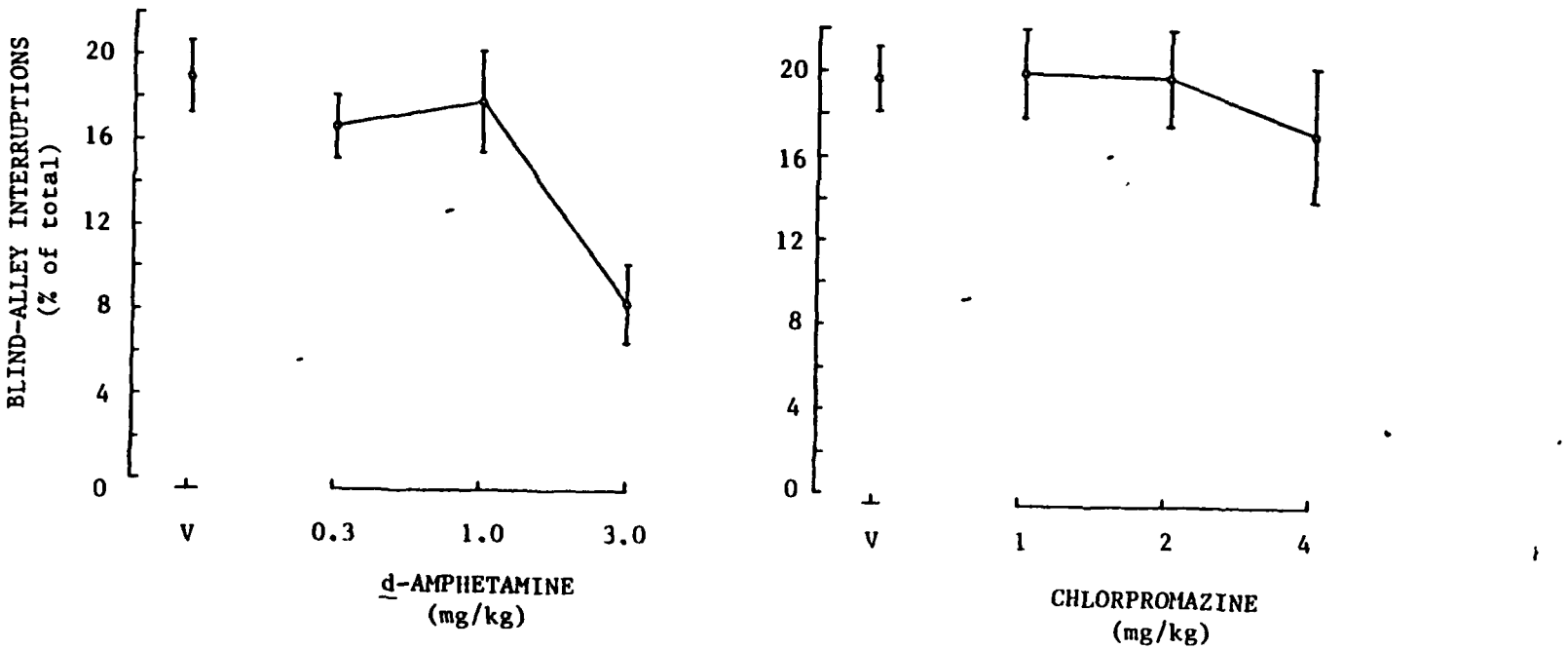

Figure 3. Drug effects on the spatial distribution of motor activity in female rats. Each symbol represents the mean (±SEM) effect of d-amphetamine or the saline vehicle (left panel), and chlorpromazine or the saline vehicle (right panel), on the number of photocell interruptions in the blind alleys of the figure-eight maze, expressed as a percentage of total interruptions.

after availability had terminated all rats were treated i.p. with either a dosage of lithium chloride, the isotonic saline vehicle or nothing. Three days later all rats were given a choice between consuming saccharin or water, and intakes were measured. The results of this experiment are shown in Figure 4. Control rats (non-injected and vehicle-injected) preferred saccharin over tap water. Lithium-treated rats, on the other hand, displayed a dosage-related aversion to saccharin (i.e., a conditioned flavor aversion). The insert to Figure 4 shows that lithium produced non-systematic effects on total fluid intake.

3. Schedule-controlled behavior. Separate groups of female rats were trained to respond under either a variable-interval 90-sec (VI 90-sec) or a variable-ratio 50-response (VR-50) schedule of milk delivery. Steady-state performance was characterized by a moderate rate of responding under VI 90-sec and a rapid rate of responding under VR-50. Once performances had stabilized, a cross-over design was used in which rats were treated p.o. with either 50 mg/kg RDX or the RDX vehicle. Several days of testing separated successive treatments owing to prominent carryover effects produced by RDX. The results of this experiment are shown in Figures 5 and 6. RDX produced a large decrease in the rate of VI-reinforced responding (Figure 5). Recovery from this effect appeared to be complete two days after dosing, although an increase in responding above baseline levels was apparent three days after dosing. RDX produced an even larger decrease in the rate of VR-reinforced responding (Figure 6). Recovery from this effect appeared to be complete by three days after dosing.

4. Analytical. The time course of RDX concentrations in plasma and brain of female rats is presented in Fig. 7. RDX was rapidly absorbed into blood and its concentration was not reduced (and if anything it

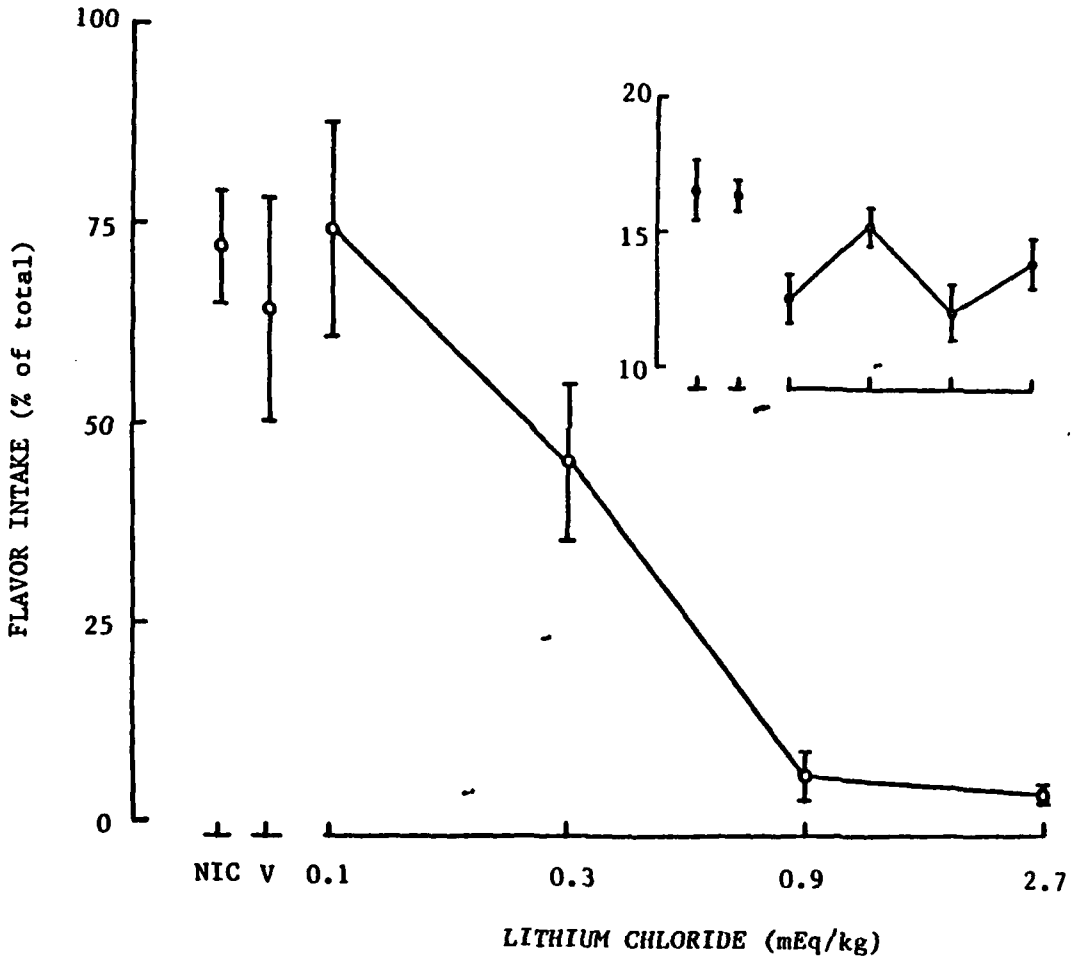

Figure 4. Flavor aversions induced by lithium chloride in female rats. Each symbol represents the mean (±SEM) saccharin intake, expressed as a percentage of total intake, of rats previously treated with a dosage of lithium chloride, the saline vehicle (V) or nothing (non-injected control, NIC) (N=6/group), when given a choice between consuming saccharin or distilled water. Insert shows comparable data for total intake.

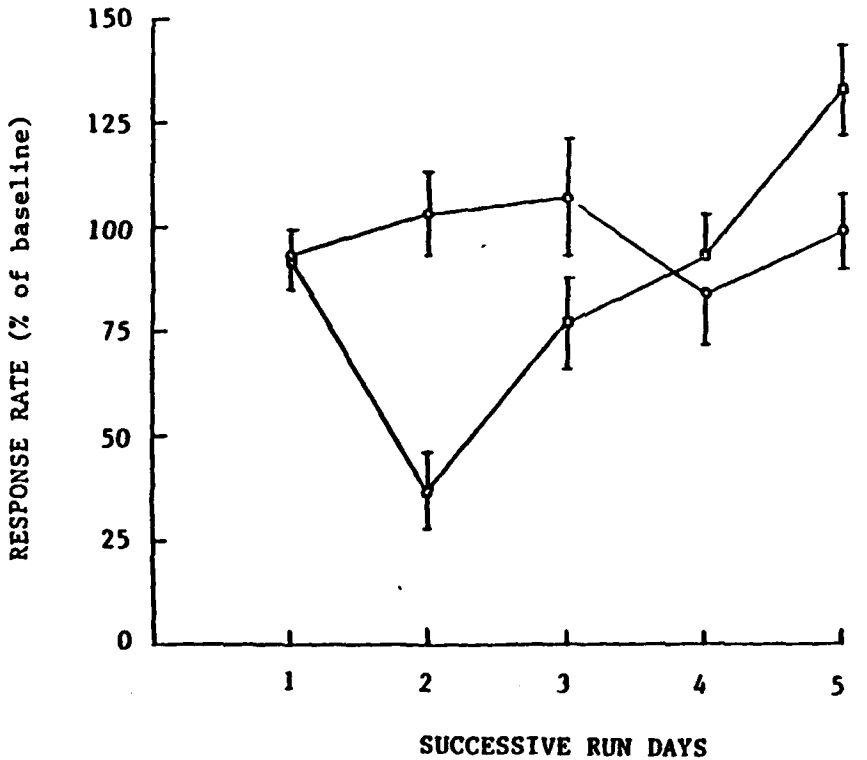

Figure 5. Effects of RDX on schedule-controlled performance. Each symbol represents the mean (±SEM) rate of response of eight female rats maintained under a variable-interval 90-sec schedule of reinforcement, expressed as a percentage of previously determined baseline response rates. Squares represent the effect of RDX (50 mg/kg) and circles represent the effect of RDX vehicle, administered prior to run day 2.

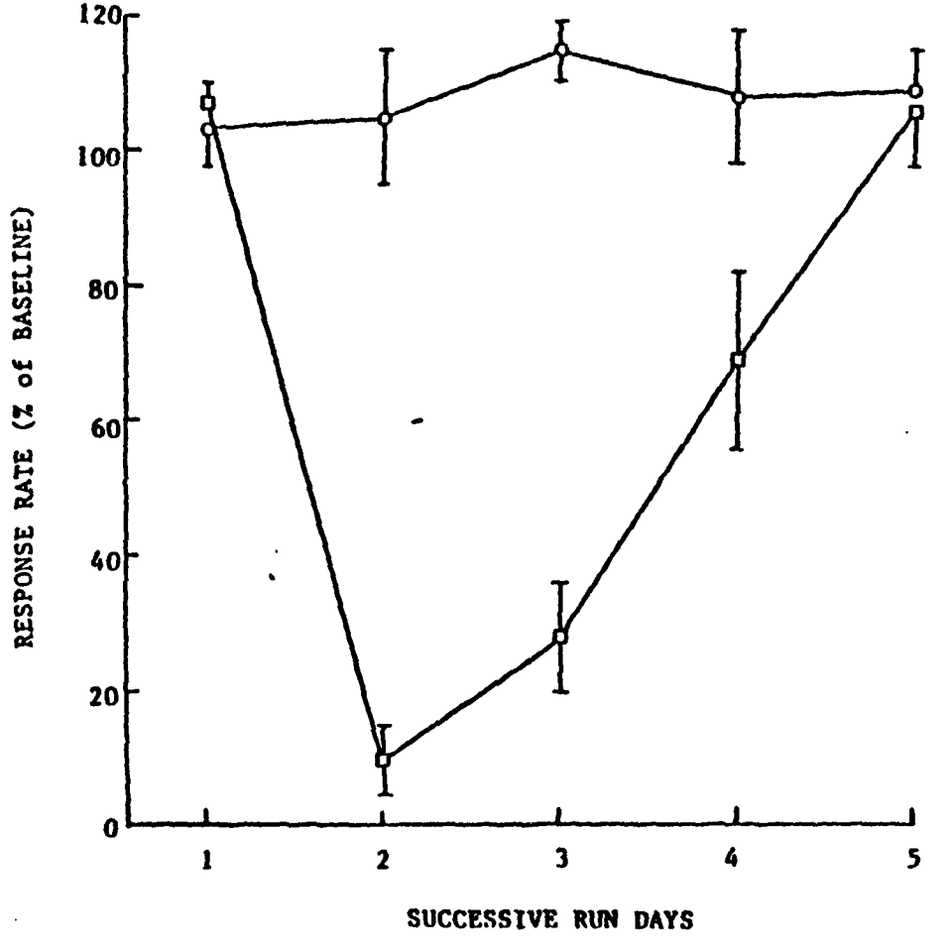

Figure 6. Effects of RDX on schedule-controlled performance. Each symbol represents the mean (±SEM) rate of response of eight female rats maintained under a variable-ratio 50-response schedule of reinforcement, expressed as a percentage of previously determined baseline response rates. Squares represent the effect of RDX (50 mg/kg) and circles represent the effect of RDX vehicle, administered prior to run day 2.

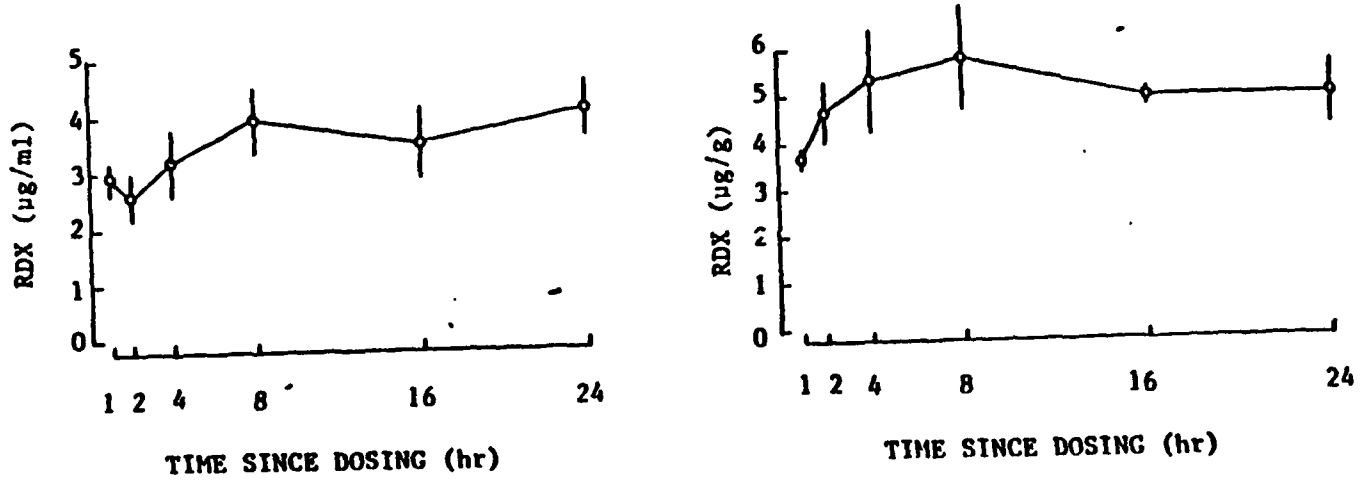

Figure 7. Time-course of RDX levels in plasma (left panel) and in brain (right panel). Adult female rats (N=4-5) were treated with RDX (50 mg/kg) and sacrificed serially at the indicated times for RDX analysis. Each symbol represents mean (±SEM) RDX concentration at each time point.

increased) during the first 24 hrs after oral dosing. RDX was similarly rapidly absorbed into brain and with no appreciable decline apparent within the first 24 hrs after dosing. Determination of the time course of RDX concentration in plasma over a longer time period using male rats revealed that the time of maximal absorption was approximately 24 hrs and that RDX was cleared from plasma by 3 days after dosing (Fig. 8). A similar relationship was observed in whole brain (Fig. 8). The time of maximal concentration in brain was approximately 24 hrs. RDX was not detectable 3 or 4 days after dosing.

B. Acute Effects of RDX.

1. Motor activity. The effects of acute RDX administration on the motor activity of male rats measured in figure-eight mazes are shown in Figure 9. RDX produced substantial dosage-related decreases in motor activity. An estimate of the ED50 was impossible to determine since even the smallest dosage produced greater than a 50% decrease in activity when compared to vehicle-control data. Redetermination of activity levels 24 hours after dosing revealed that RDX still produced noticeable dosage-related decreases in motor activity.

In addition to decreasing overall levels of motor activity, RDX altered the within-session relative distribution of motor activity (see Figure 10). Vehicle-treated control rats displayed a progressive decrease in motor activity throughout the one-hour test session. RDX appeared to accentuate the within-session decay in activity, and in a dosage-related fashion, although all dosages appeared to produce similar effects on the asymptotic level of motor activity obtained late in the session. Some evidence of a residual effect of RDX was apparent 24 hours after dosing, especially at the largest dosage (Figure 10).

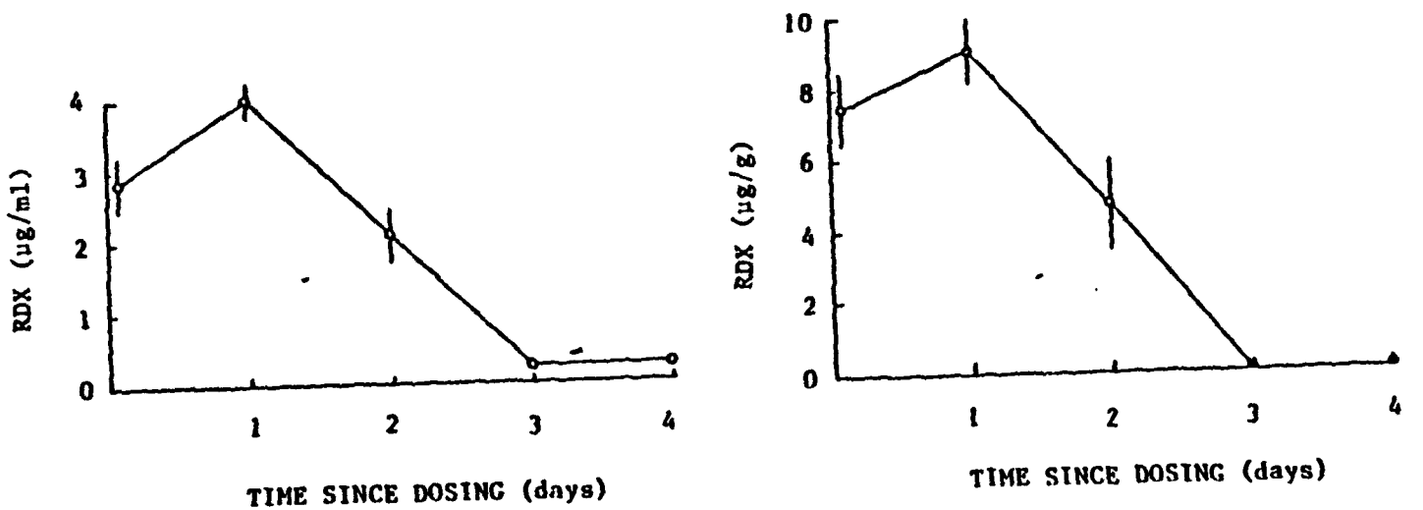

Figure 8. Time-course of RDX levels in plasma (left panel) and in brain (right panel). Adult rats (N=6/group) were treated with RDX (50 mg/kg) and sacrificed at the indicated times for RDX analysis. Each symbol represents mean (±SEM) RDX concentration at each time point.

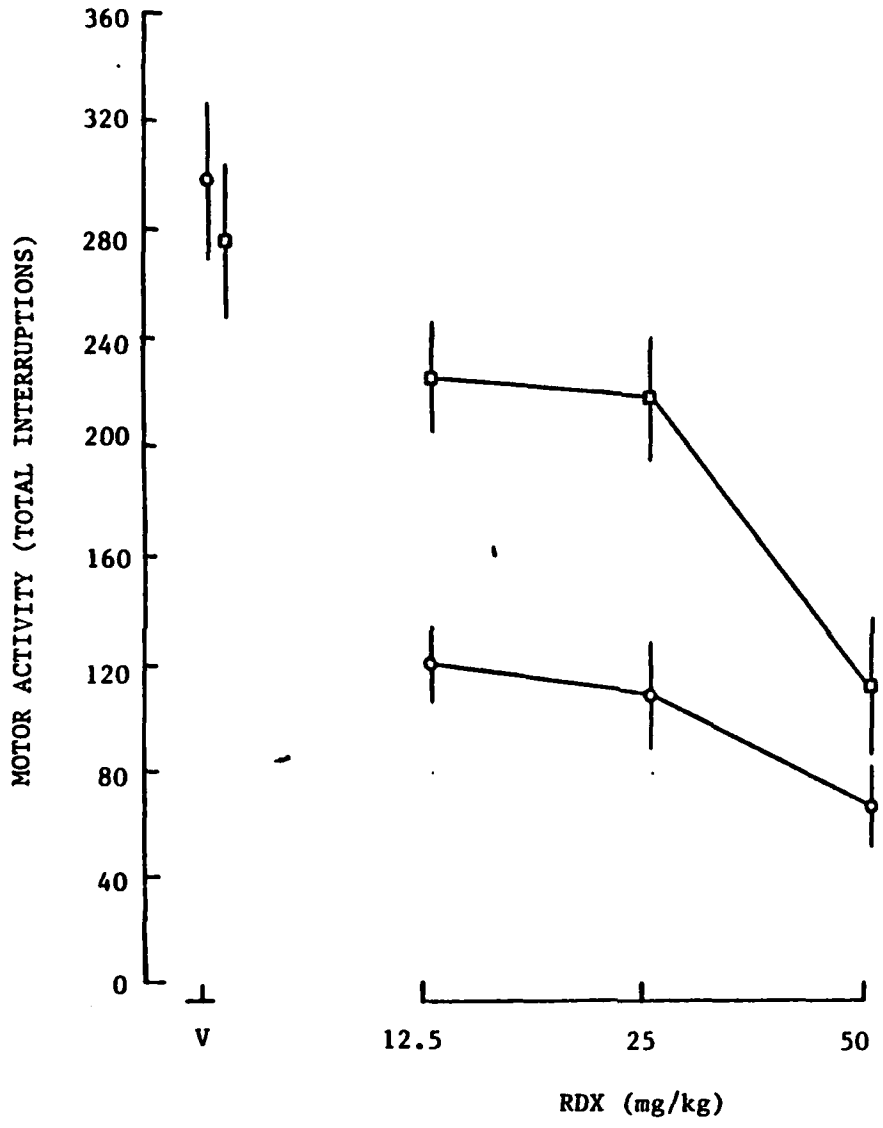

Figure 9. Acute effects of RDX on motor activity in male rats. Each symbol represents the mean (±SEM) effect of RDX or RDX vehicle (N=8/group) on total photocell interruptions recorded in figure-eight mazes during a one-hour session. Circles represent effects obtained 2 hours after dosing and squares represent effects obtained 24 hours after dosing.

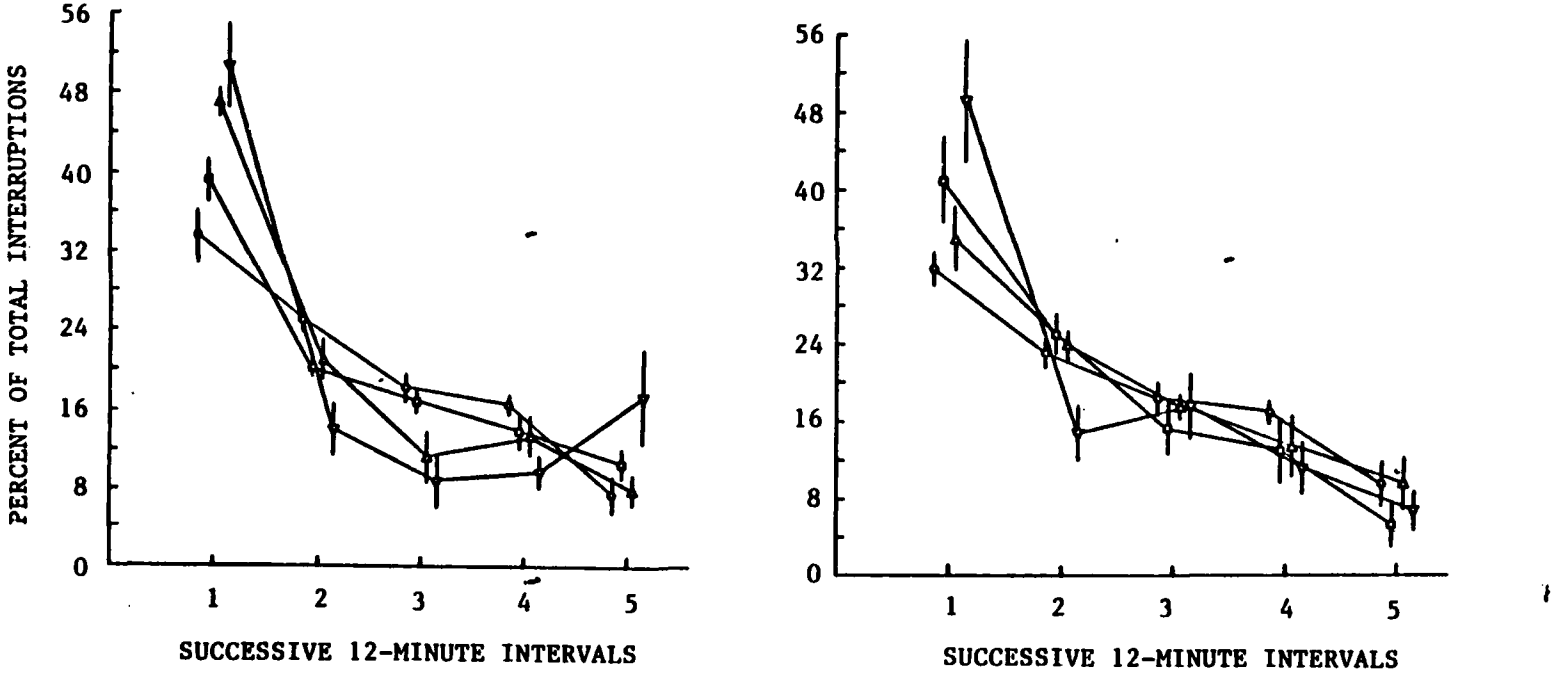

Figure 10. Acute effects of RDX on the within-session distribution of motor activity in male rats. Each symbol represents the mean (±SEM) effect of RDX (squares, 12.5 mg/kg; triangles, 25 mg/kg; inverted triangles, 50 mg/kg) or RDX vehicle (circles) (N=8/group) on motor activity, expressed as a percentage of total activity, across successive 12-min periods of the one-hour test session. Left panel represents effects obtained 2 hours after dosing and right panel represents effects obtained 24 hours after dosing. Symbols have been displaced for ease of visual representation.

RDX also appeared to modify the spatial distribution of motor activity. This effect is shown in Figure 11, in which the percentage of total photocell interruptions occurring in the blind alleys of the figure-eight maze is shown following treatment with either vehicle or RDX. On the day of dosing, vehicle-treated control rats displayed a less-than-chance likelihood of photocell interruptions in the blind alleys (an equal distribution of motor activity in all portions of the maze would be shown as 25% of total interruptions occurring in the blind alleys). RDX further accentuated this tendency to avoid the blind alleys, although the dosage dependence was not as great as that seen for changes in overall levels of motor activity (see Figure 9). A residual effect of RDX was apparent 24 hours after dosing, when compared to vehicle-control data.

2. Landing footspread. The same rats used to evaluate the acute effects of RDX on motor activity were also used shortly thereafter on the day of dosing to measure landing footspread. RDX consistently and significantly ($F=5.71$, $df=3,28$, $P<0.005$) decreased hindlimb landing footspread, when compared to vehicle-control data, but not in a dosage-related fashion (Figure 12).

3. Flavor-aversion conditioning. Pairing consumption of a distinctly flavored solution (saccharin) with administration of RDX produced substantial aversions to saccharin when the rats were subsequently given a choice between consuming it and tap water (see Figure 13). Vehicle-treated rats on the other hand consistently preferred saccharin under the same conditions of testing. The data displayed in Figure 13 were obtained 24 hours after dosing, and although the conditioned flavor aversion was not dosage related, RDX did produce a substantial dosage-related decrease in total fluid intake (see insert of Figure 13). When given a second choice between consuming saccharin and tap water (Figure 14), vehicle-treated rats still preferred

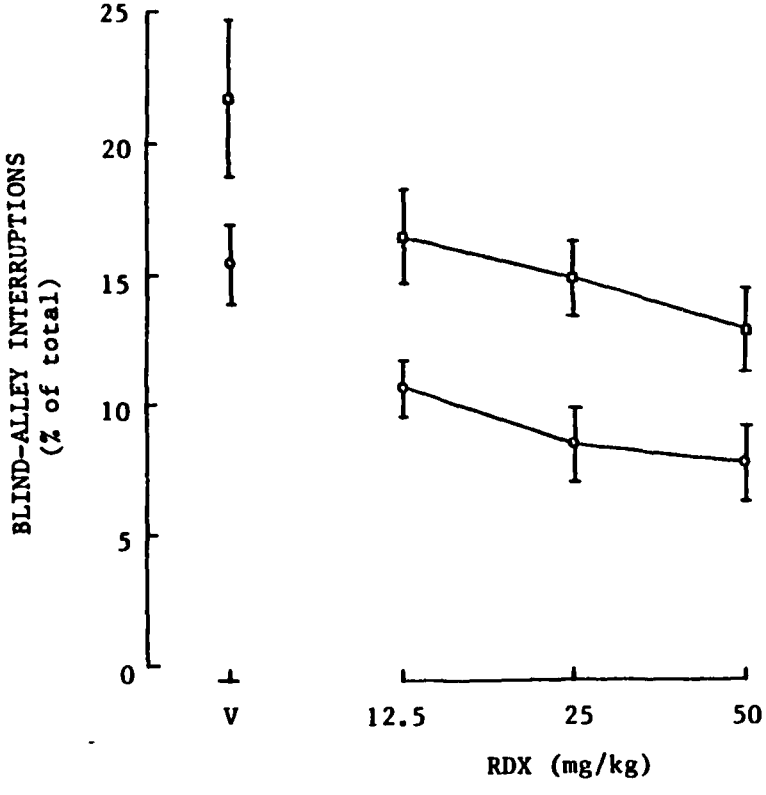

Figure 11. Acute effects of RDX on the spatial distribution of motor activity in male rats. Each symbol represents the mean (+SEM) effect of RDX or RDX vehicle (N=8/group) on the number of photocell interruptions in the blind alleys of the figure-eight maze, expressed as a percentage of total interruptions. Circles represent effects obtained 2 hours after dosing and squares represent effects obtained 24 hours after dosing.

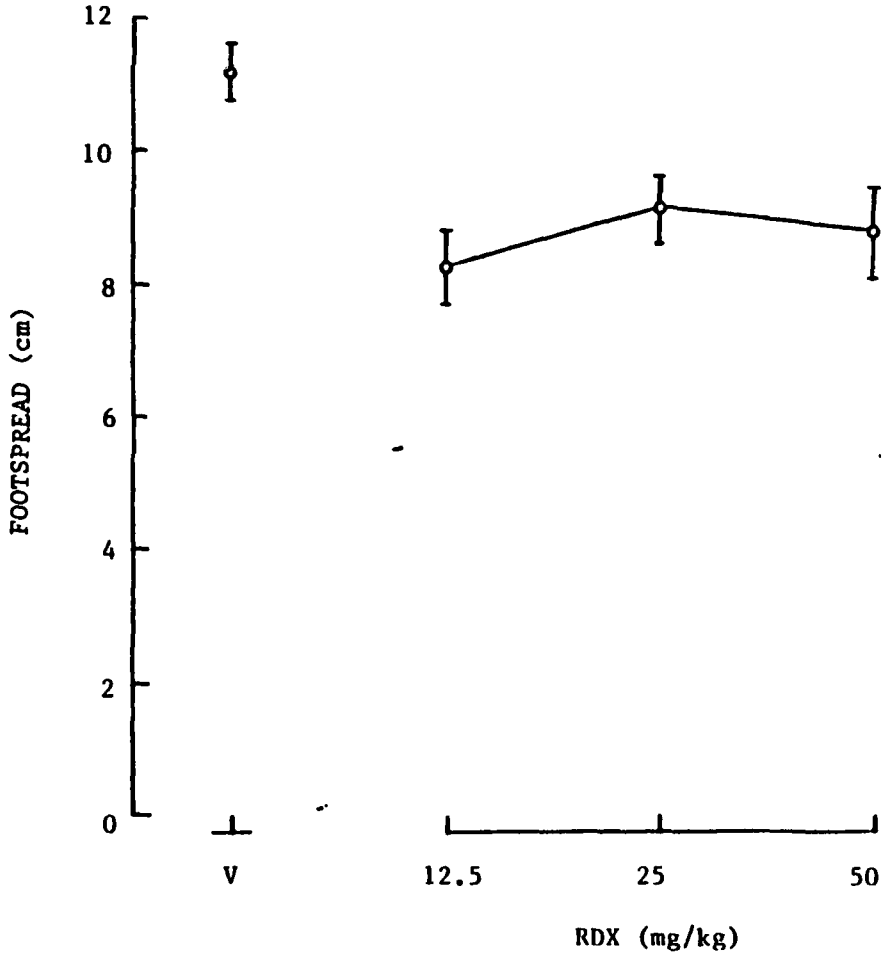

Figure 12. Acute effects of RDX on landing footspread in male rats. Each symbol represents the mean (+SEM) effect of RDX or RDX vehicle (N=8/group) on hindlimb splay (in cm). All rats were tested immediately after a one-hour session in the figure-eight mazes.

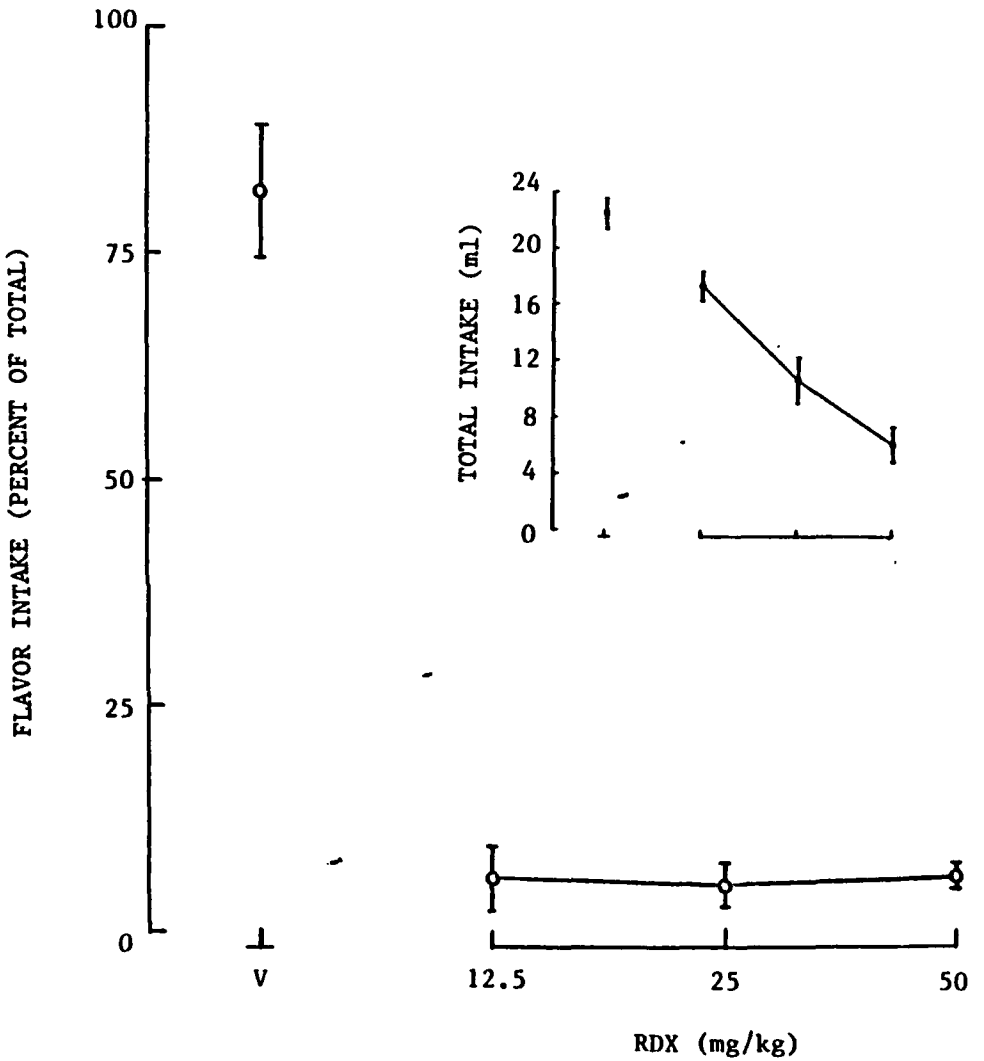

Figure 13. Flavor aversions induced by acute RDX administration. Each symbol represents the mean (±SEM) saccharin intake, expressed as a percentage of total intake, of male rats previously receiving access to saccharin followed by either RDX or RDX vehicle (N=9/group), when given a choice between saccharin and distilled water. Choice data were collected 24 hours after pairing saccharin availability with RDX administration. Insert shows treatment effects on total intake.

saccharin whereas RDX-treated rats still preferred tap water. The only difference between the data portrayed in Figures 13 and 14 was that (1) the effect of the smallest dosage was intermediate to that of both the vehicle and the larger dosages of RDX and (2) there were no consistent effects of RDX on total fluid intake (see insert of Figure 14).

4. Schedule-controlled behavior. Male rats were trained to respond under a variable-interval 90-sec (VI 90-sec) or a variable-ratio 50-response (VR-50) schedule of milk reinforcement. Once performances had stabilized, the acute effects of RDX were determined, and are shown in Figure 15. RDX produced substantial reductions in overall rates of responding under both VI 90-sec and VR-50. Slightly greater effects were produced by RDX on VR-reinforced responding, especially at the largest dosage. For performance under either schedule, however, the effects of the two largest dosages of RDX did not differ from each other.

Figure 16 shows the time course for recovery from the acute effects of RDX on VI-reinforced responding. The time required to recover baseline performance generally increased with increasing dosage of RDX; complete recovery was achieved three days after dosing. Comparable data for recovery from the acute effects of RDX on VR-reinforced responding are shown in Figure 17. As with VI-reinforced responding, the time required to recover baseline performance was an increasing function of RDX dosage. Recovery was generally achieved by three days after dosing, although a residual effect of the largest dosage was still somewhat apparent at this time.

The acute effects of RDX and the time course of recovery were also determined for the performance of female rats tested under comparable conditions. Figure 18 shows the acute effects of RDX on schedule-controlled responding. RDX produced substantial dosage-related decreases in responding under both

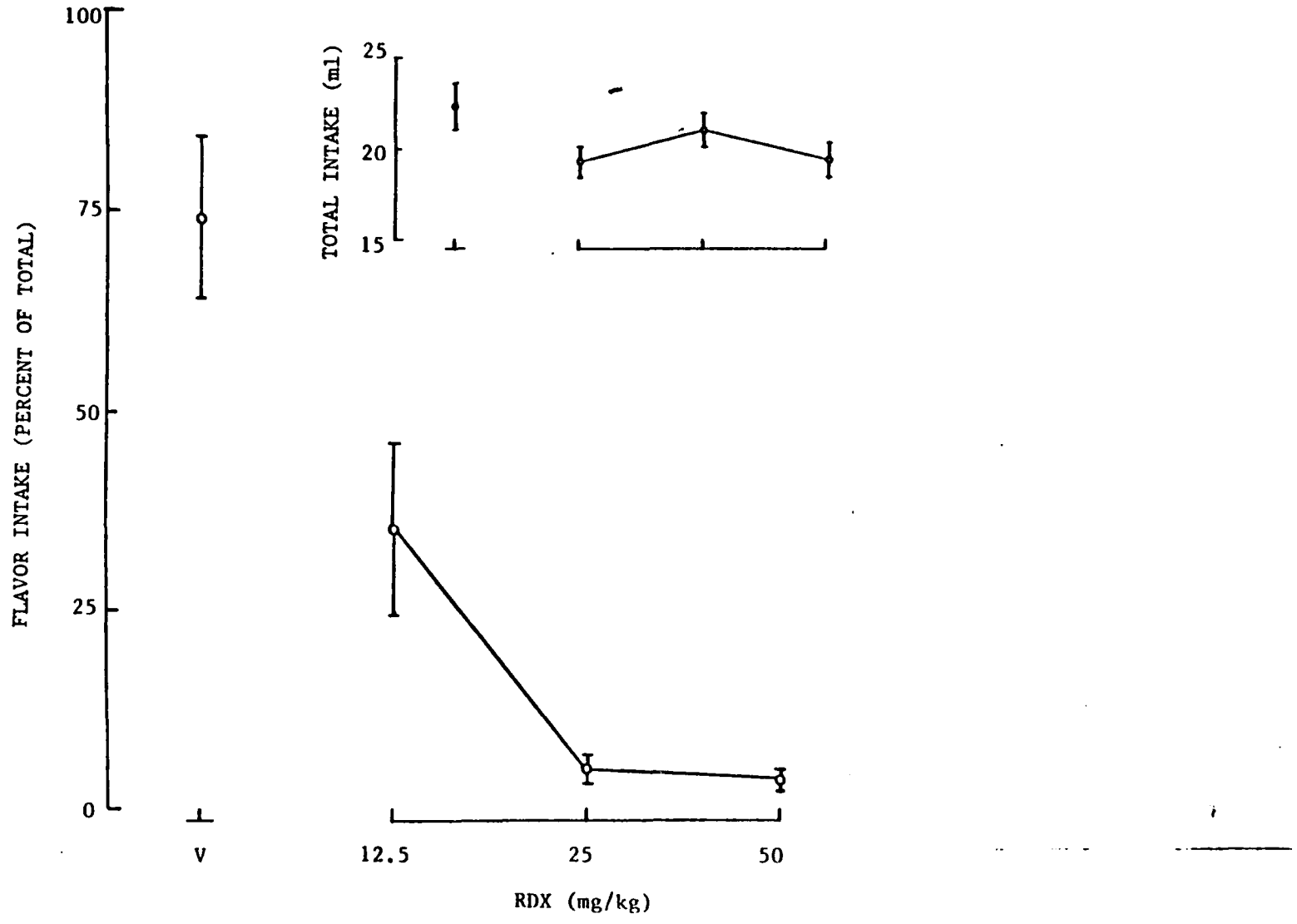

Figure 14. Flavor aversions induced by acute RDX administration. Each symbol represents the mean (±SEM) saccharin intake, expressed as a percentage of total intake, of male rats previously receiving access to saccharin followed by either RDX or RDX vehicle (N=9/group), when given a choice between saccharin and distilled water. Choice data were collected 72 hours after pairing saccharin availability with RDX administration. Insert shows treatment effects on total intake.

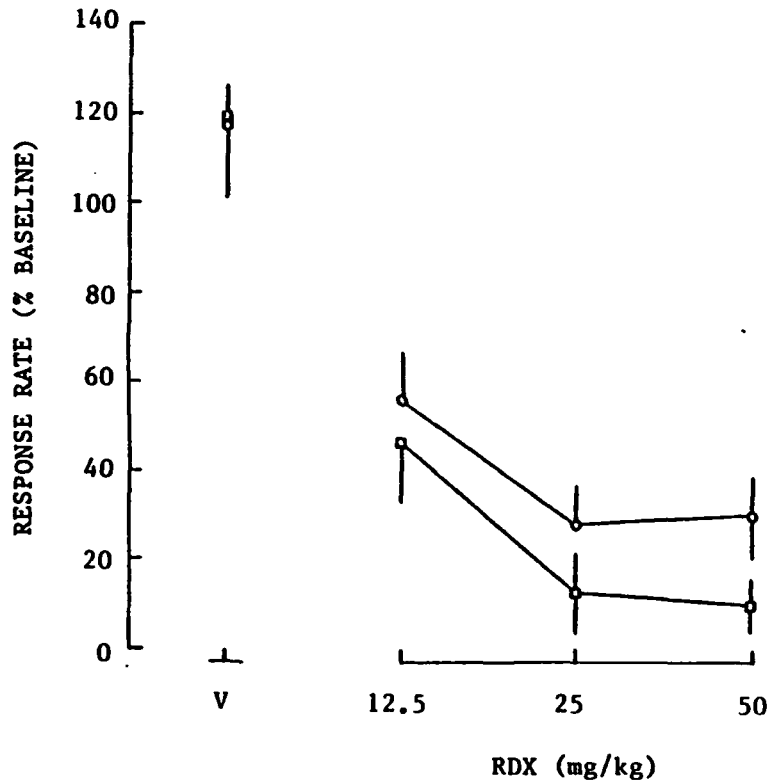

Figure 15. Acute effects of RDX on schedule-controlled behavior in male rats. Each symbol represents the mean (±SEM) rate of responding (expressed as a percentage of previously determined baseline rates) following administration of RDX or RDX vehicle (N=8/group), under a variable-interval 90-sec (circles) or a variable-ratio 50-response (squares) schedule.

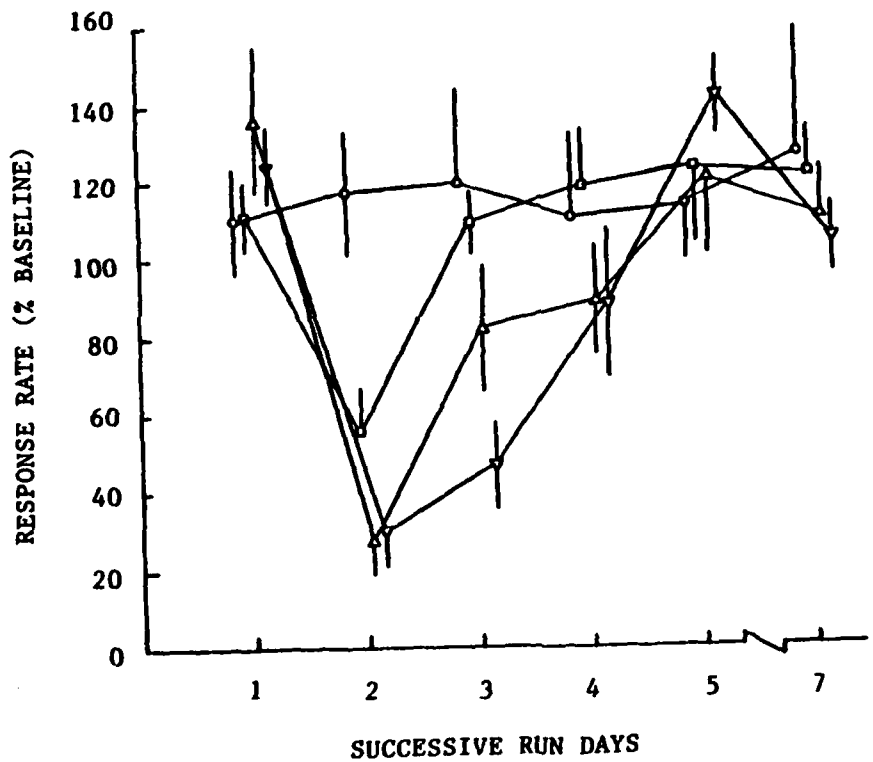

Figure 16. Recovery from the acute effects of RDX on schedule-controlled performance. Male rats (N=8/group), previously trained to perform under a variable-interval 90-sec schedule, were treated with either RDX (squares, 12.5 mg/kg; triangles, 25 mg/kg; inverted triangles, 50 mg/kg) or RDX vehicle (circles) prior to run day 2. Each symbol represents the mean (±SEM) effect obtained on this and subsequent run days, expressed as a percentage of previously determined baseline rates. Symbols have been displaced for ease of visual representation.

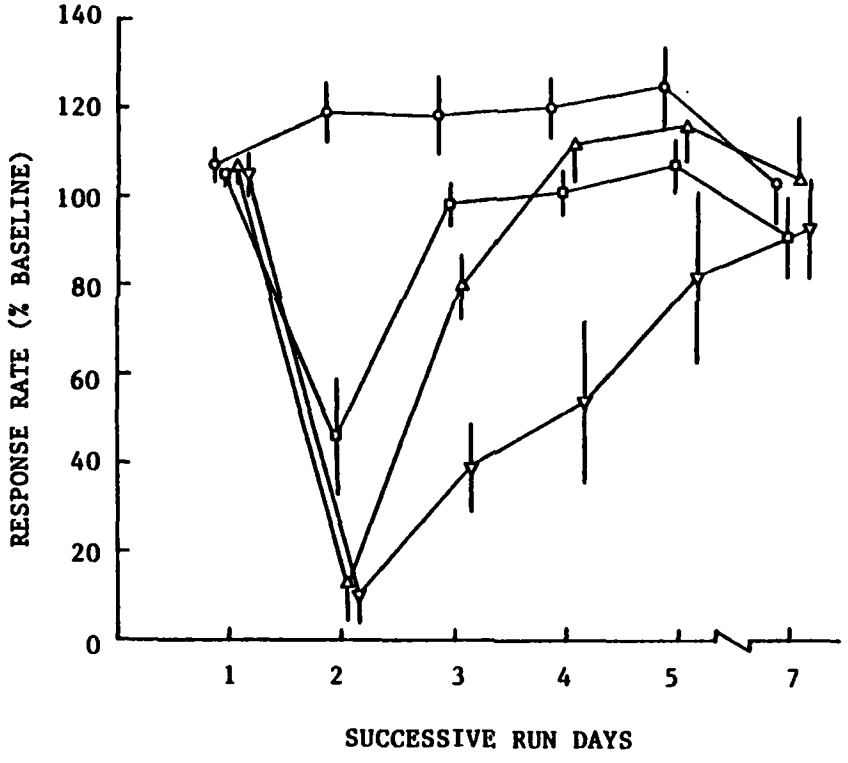

Figure 17. Recovery from the acute effects of RDX on schedule-controlled performance. Male rats (N=8/group), previously trained to perform under a variable-ratio 50-response schedule, were treated with either RDX (squares, 12.5 mg/kg; triangles, 25 mg/kg; inverted triangles, 50 mg/kg) or RDX vehicle (circles) prior to run day 2. Each symbol represents the mean (±SEM) effect obtained on this and subsequent run days, expressed as a percentage of previously determined baseline rates. Symbols have been displaced for ease of visual representation.

schedules, although the magnitude of the decreases was consistently greater under VR-50 than under VI 90-sec. Figure 19 shows the time course for recovery from the effects of RDX on VI-reinforced responding. The time required to recover baseline performance generally increased with increasing dosage. A "rebound" increase in response rate above baseline levels was apparent in rats that had received 25 mg/kg, but not in rats that had received smaller or larger RDX dosages. Recovery appeared to be complete in all groups by 5 days post-dosing. Comparable data for the recovery of VR-reinforced responding are shown in Figure 20. The time required to recover baseline performance was a direct function of RDX dosage, and recovery appeared to be complete in all groups by 4 days post-dosing.

5. Acoustic startle response. Figure 21 shows that acute RDX administration produced dosage-related decreases in startle amplitude at all background noise intensities. In addition, this figure shows that RDX decreased the sensitization (enhancement) of startle amplitude produced by increasing background noise intensity. Figure 21 also shows the effect of RDX on startle response latency. RDX increased response latency in a dosage-related fashion; these increases in latency were greatest at the lowest background noise condition even though latency did not vary with noise condition in vehicle-treated rats.

6. Analytical. RDX concentrations in both plasma and brain were to a first approximation linearly related to dosage when determined approximately 3 hrs after dosing (Fig. 22). These data show that RDX was readily absorbed into both blood and brain and that, when compared on a parts-per-million basis, brain concentrations of RDX were greater than those in plasma. This latter relationship was also apparent in the RDX time-course data shown in Figures 7 and 8.

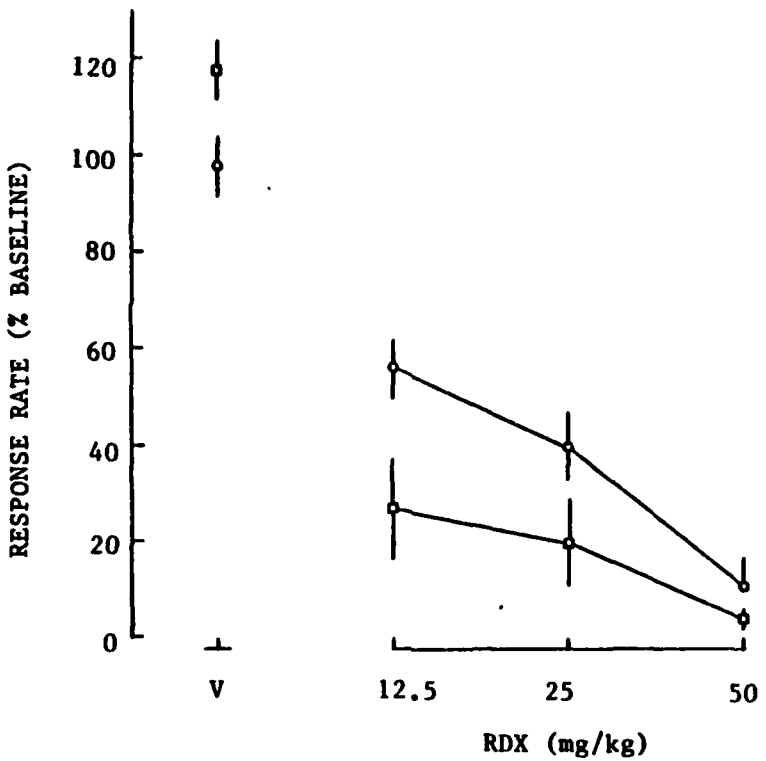

Figure 18. Acute effects of RDX on schedule-controlled behavior in female rats. Each symbol represents the mean (±SEM) rate of responding (expressed as a percentage of previously determined baseline rates) following administration of RDX or RDX vehicle (N=8/group), under a variable-interval 90-sec (circles) or a variable-ratio 50-response (squares) schedule.

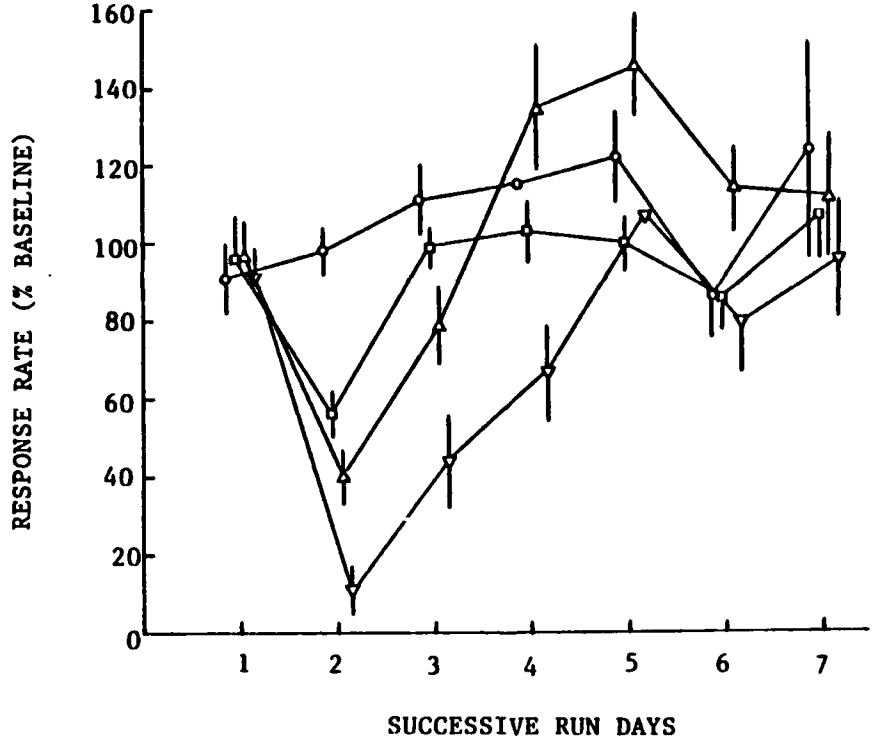

Figure 19. Recovery from the acute effects of RDX on schedule-controlled performance. Female rats (N=8/group), previously trained to perform under a variable-interval 90-sec schedule, were treated with either RDX (squares, 12.5 mg/kg; triangles, 25 mg/kg; inverted triangles, 50 mg/kg) or RDX vehicle (circles) prior to run day 2. Each symbol represents the mean (±SEM) effect obtained on this and subsequent run days, expressed as a percentage of previously determined baseline rates. Symbols have been displaced for ease of visual representation.

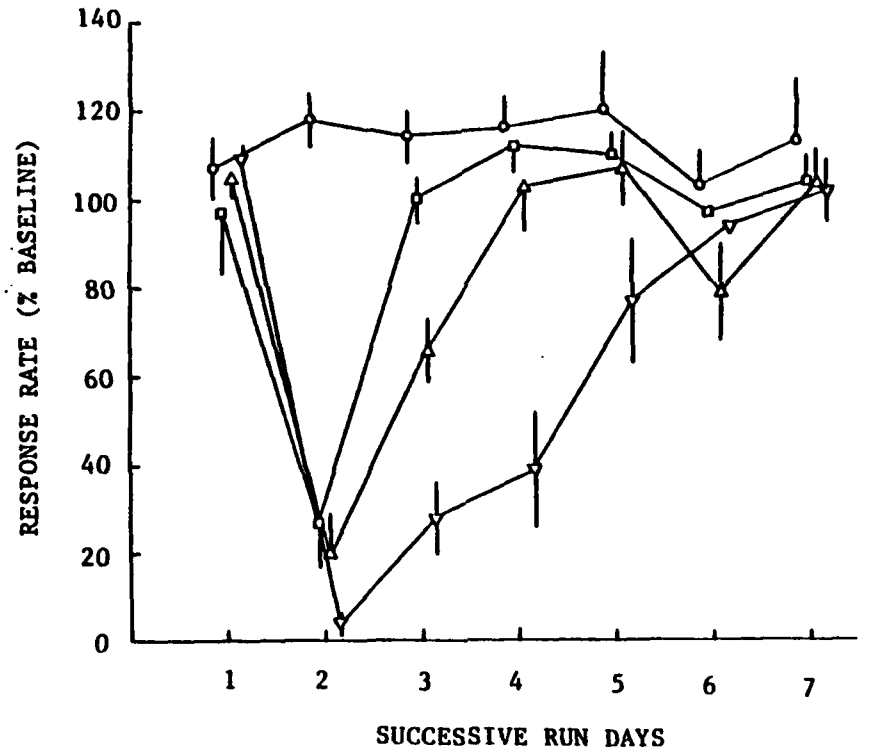

Figure 20. Recovery from the acute effects of RDX on schedule-controlled performance. Female rats (N=8/group), previously trained to perform under a variable-ratio 50-response schedule, were treated with either RDX (squares, 12.5 mg/kg; triangles, 25 mg/kg; inverted triangles, 50 mg/kg) or RDX vehicle (circles) prior to run day 2. Each symbol represents the mean (±SEM) effect obtained on this and subsequent run days, expressed as a percentage of previously determined baseline rates. Symbols have been displaced for ease of visual representation.

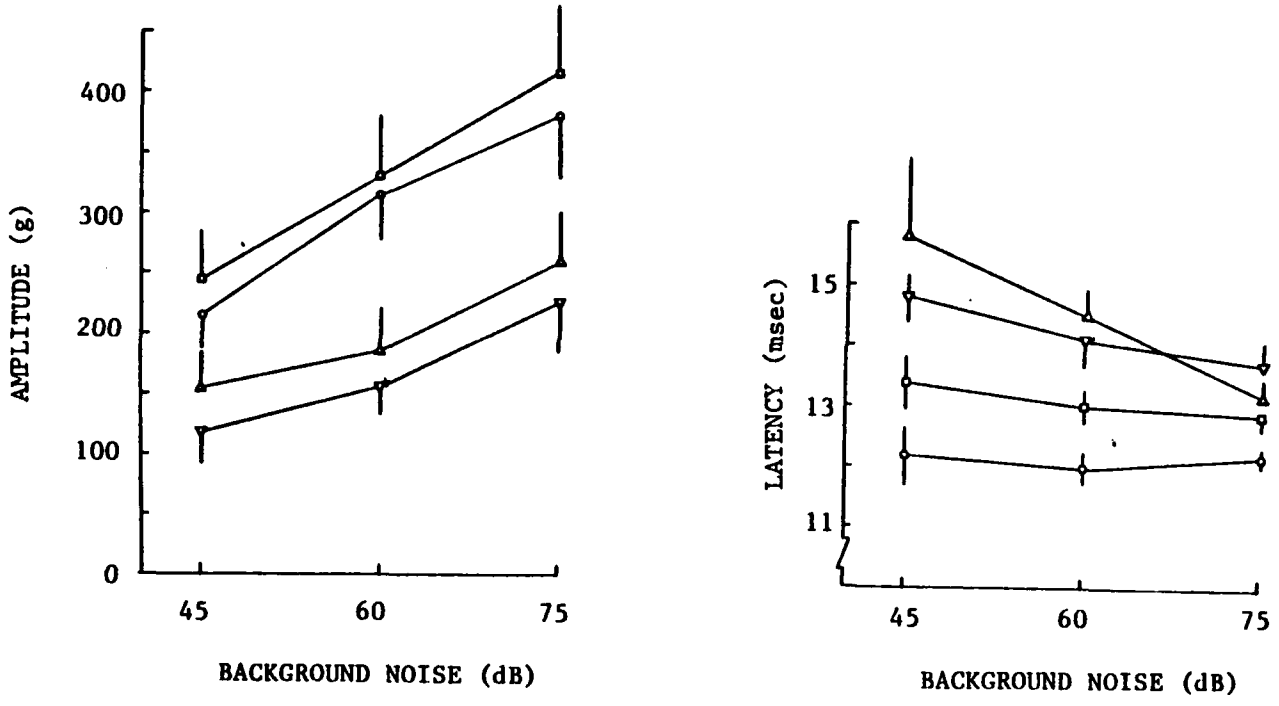

Figure 21. Acute effects of RDX on the acoustic startle response in male rats. Left panel shows mean (±SEM) amplitude and right panel shows mean (±SEM) latency of the startle response, plotted as a function of background noise intensity for RDX-treated (squares, 12.5 mg/kg; triangles, 25 mg/kg; inverted triangles, 50 mg/kg) and vehicle-treated (circles) rats (N=10/group).

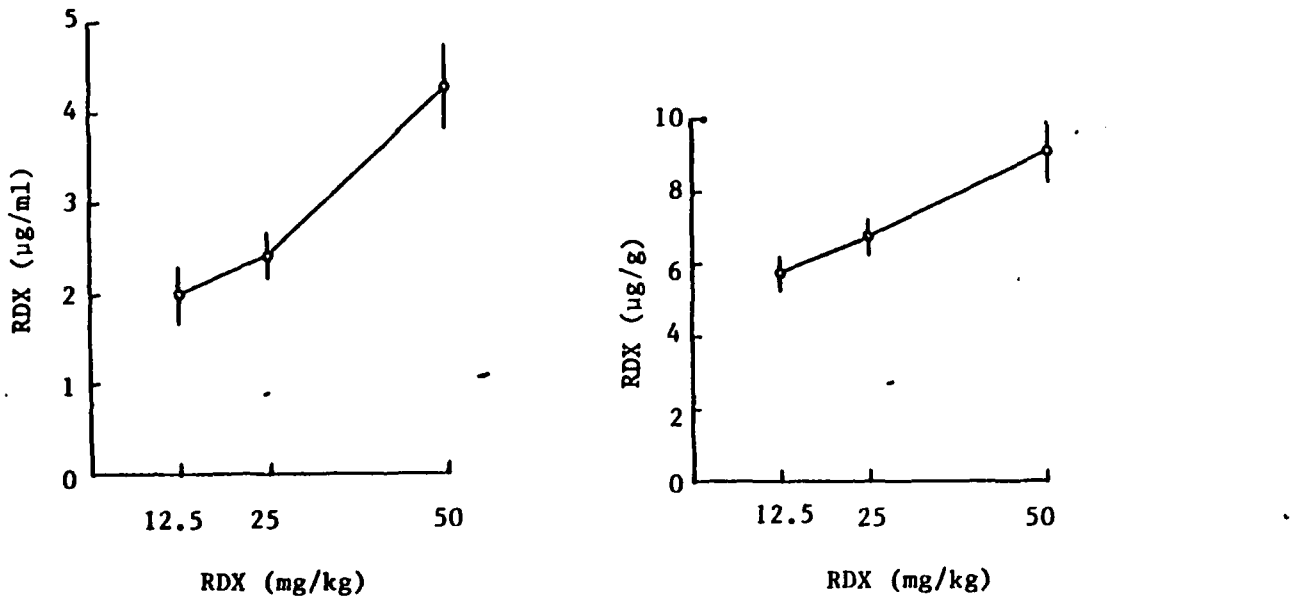

Figure 22. RDX concentrations in plasma (left panel) and in brain (right panel), determined approximately 3 hr after dosing. Each symbol represents mean (±SEM) RDX concentrations at each dosage of RDX administered (N=10 male rats/group).

C. Subchronic Effects of RDX

1. Motor activity. Male rats were arbitrarily divided into groups designated to receive 30-day exposures to RDX (1, 3 or 10 mg/kg) or the RDX vehicle. Testing occurred on the day preceding the sub-chronic dosing regimen and on day 16 and 31 of the regimen. The effects of RDX on overall motor activity levels are shown in Figure 23. On the day preceding initiation of the subchronic regimen levels of motor activity were roughly comparable between all four groups of rats, and non-systematically related to their designated treatments (left panel). On day 16 of dosing (i.e., one day after the 15th daily dose but before the 16th treatment), small decreases in motor activity were generally obtained in all groups of RDX-treated rats but were not systematically related to the daily dosage of RDX being received (center panel). On day 31 (i.e., one day after the 30th daily treatment), activity levels were again generally lower than those obtained in control rats, and non-systematically related to RDX dosage (right panel). Comparison of the day 16 and day 31 data indicate that the magnitude of the decreases in motor activity was generally smaller on day 31 than on day 16. An analysis of variance revealed, however, that there was no statistically reliable effect of subchronic RDX treatment, test time (day 16 vs. 31) or treatment-by-time interaction.

The effects of subchronic RDX administration on the within-session distribution of motor activity are shown in Figure 24. Prior to initiating the dosing regimen, all rats displayed a prominent within-session decay of motor activity. Groups designated to receive RDX displayed more prominent decay curves when compared to the group designated to receive vehicle (left panel), although there were no systematic differences between the groups designated to receive the various dosages of RDX. Slight differences

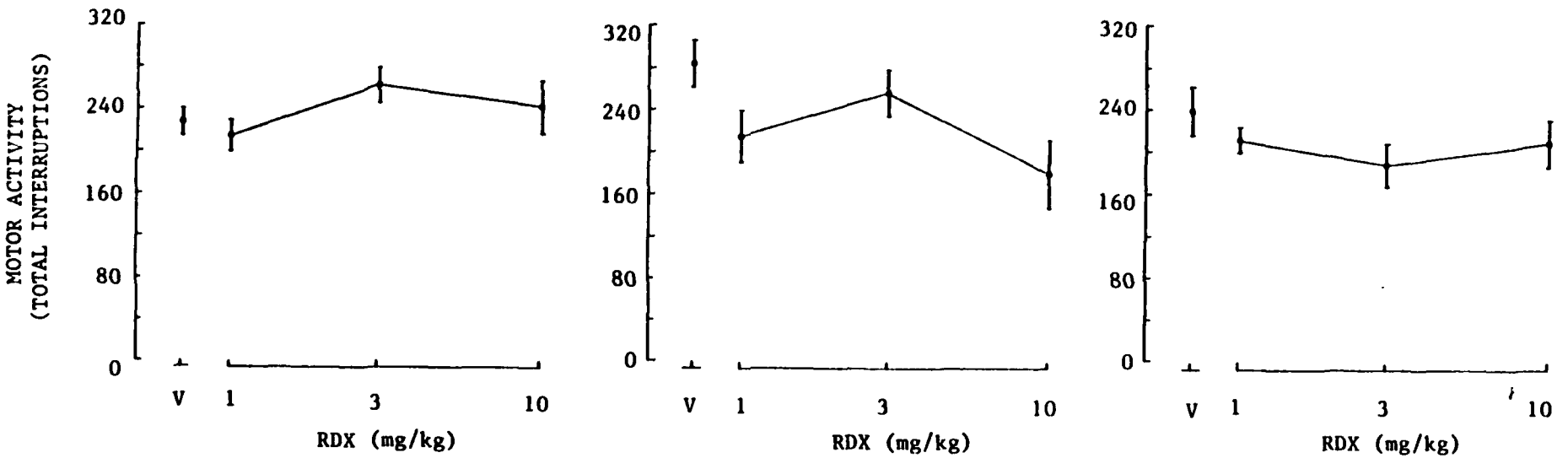

Figure 23. Subchronic effects of RDX on motor activity in male rats. Each symbol represents the mean (±SEM) effect of RDX or RDX vehicle (N=8/group) on total photocell interruptions recorded in figure-eight mazes during one-hour sessions, occurring the day before the subchronic regimen (left panel), and on the day after either the 15th (center panel) or 30th (right panel) daily treatment.

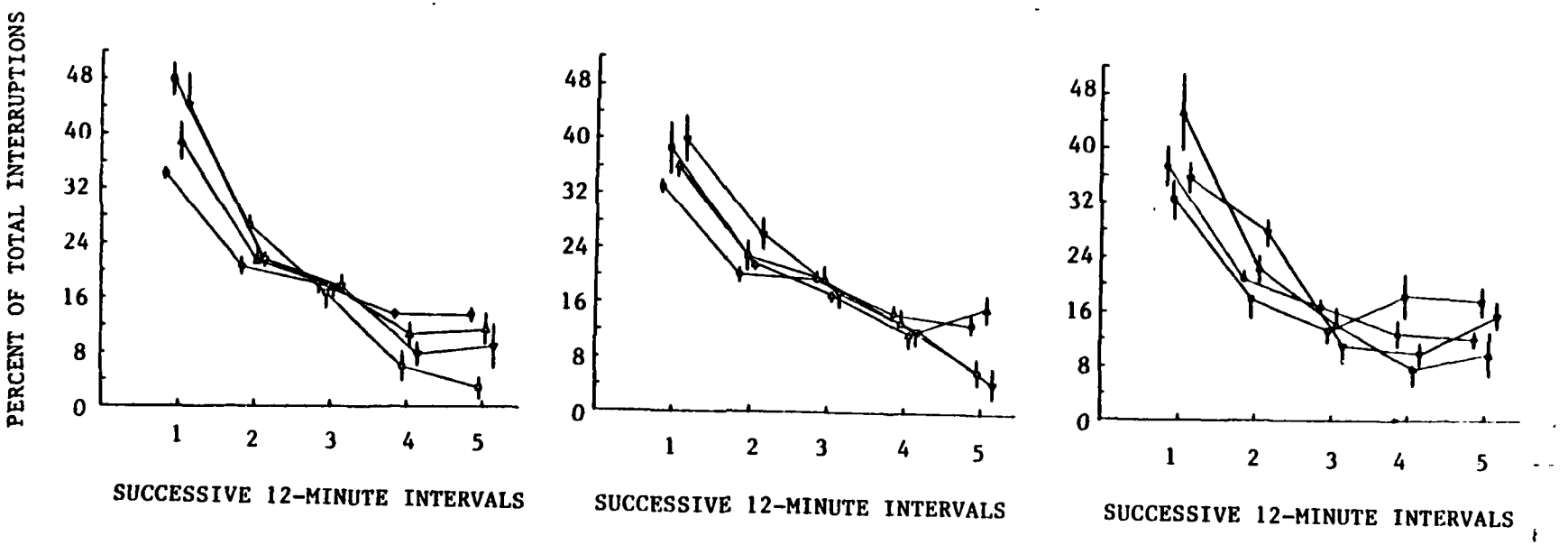

Figure 24. Subchronic effects of RDX on the within-session distribution of motor activity. Each symbol represents the mean (+SEM) effect of RDX (squares, 1 mg/kg; triangles, 3 mg/kg; inverted triangles, 10 mg/kg) or RDX vehicle (circles) (N=8/group) on motor activity in male rats, expressed as a percentage of total activity, across successive 12-min periods of the one-hour test session. Testing occurred on the day before the subchronic regimen (left panel), as well as on the day after the 15th (center panel) and the 30th (right panel) daily treatments. Symbols have been displaced for ease of visual representation.

in the decay of activity were also seen on day 16, but were generally not related to daily RDX treatment (center panel). On day 31, no differences in the within-session distribution of motor activity were apparent between vehicle-treated and RDX-treated rats (right panel).

The effects of subchronic RDX administration on the spatial distribution of motor activity are shown in Figure 25. No consistent effects of RDX were obtained that could be related either to the dosage of RDX administered or to the length of time RDX was administered.

2. Landing footspread. The effects of subchronic RDX administration on landing footspread are shown in Figure 26. RDX did not affect landing footspread after either the 15th or 30th daily dose.

3. Flavor-aversion conditioning. Rats received restricted daily access (30 min/day) to deionized water prior to and throughout the 30-day subchronic dosing regimen. Daily water intakes for each treatment group throughout the dosing regimen are shown in Figure 27. RDX produced no discernible effect on daily water intake. On day 31 all rats received 30-min access to saccharin solution, followed 20-min later by an i.p. injection of either saline or lithium chloride (0.9 mEq/kg). Figure 28 shows that there was no effect on saccharin intake that could be systematically related to subchronic RDX treatment. Three days after pairing saccharin intake with either saline or lithium chloride administration, all rats were given a 30-min choice between consuming saccharin or water. The results of this experiment are shown in Figure 29. Rats receiving saline after saccharin consistently preferred saccharin over water, regardless of their dosing history. Rats receiving lithium chloride after saccharin , on the other hand, consistently preferred water; that is, conditioned flavor aversions were established in all groups of

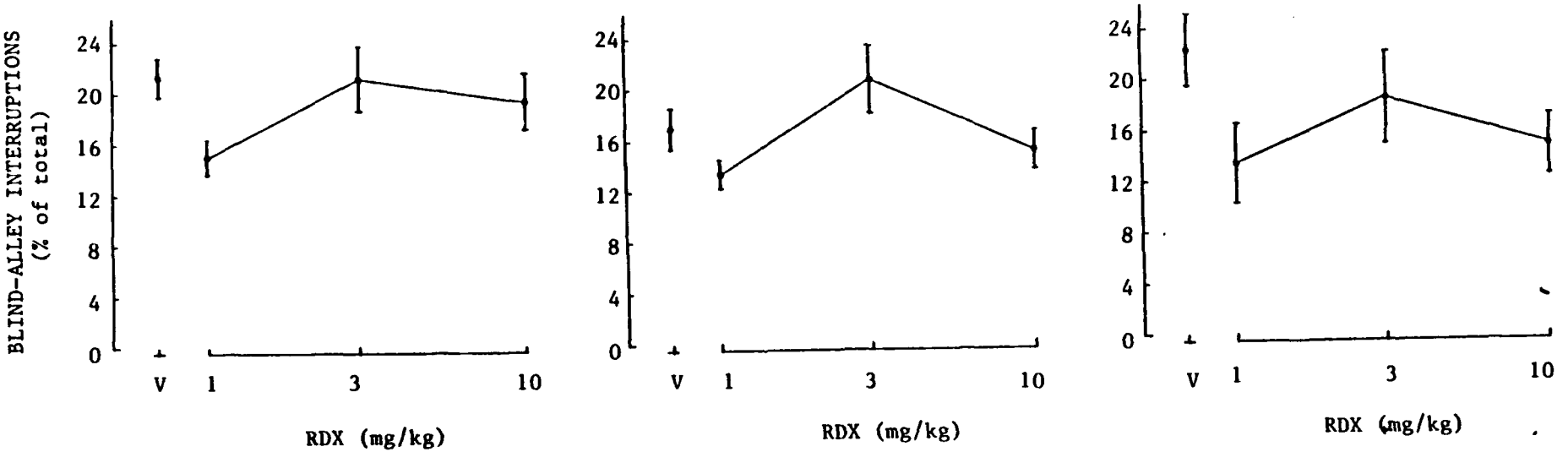

Figure 25. Subchronic effects of RDX on the spatial distribution of motor activity in male rats. Each symbol represents the mean (+SEM) effect of RDX (squares, 1 mg/kg; triangles, 3 mg/kg; inverted triangles, 10 mg/kg) or RDX vehicle (circles) (N=8/group) on the number of photocell interruptions in the blind alleys of the figure-eight maze, expressed as a percentage of total interruptions. Testing occurred on the day before the subchronic regimen (left panel), as well as on the day after the 15th (center panel) and the 30th (right panel) daily treatments.

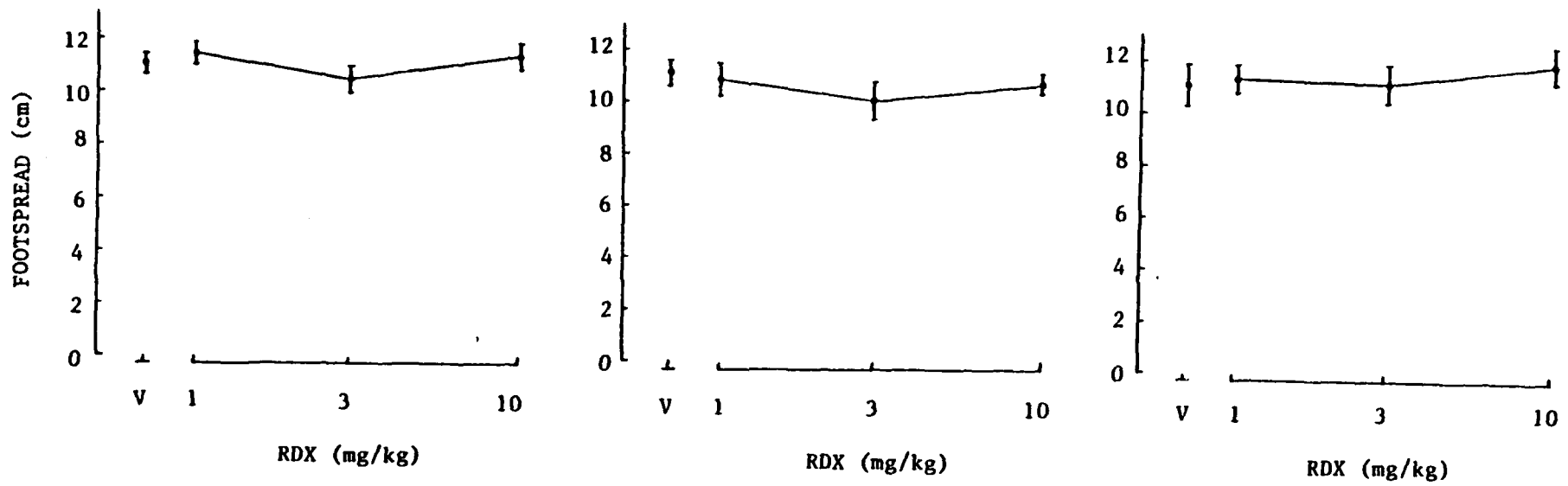

Figure 26. Subchronic effects of RDX on landing footspread in male rats. Each symbol represents the mean (±SEM) effect of RDX or RDX vehicle (N=8/group) on hindlimb splay (in cm). All rats were tested immediately after a one-hour session in the figure-eight mazes. Testing occurred on the day before the subchronic regimen (left panel), as well as on the day after the 15th (center panel) and the 30th (right panel) daily treatments.

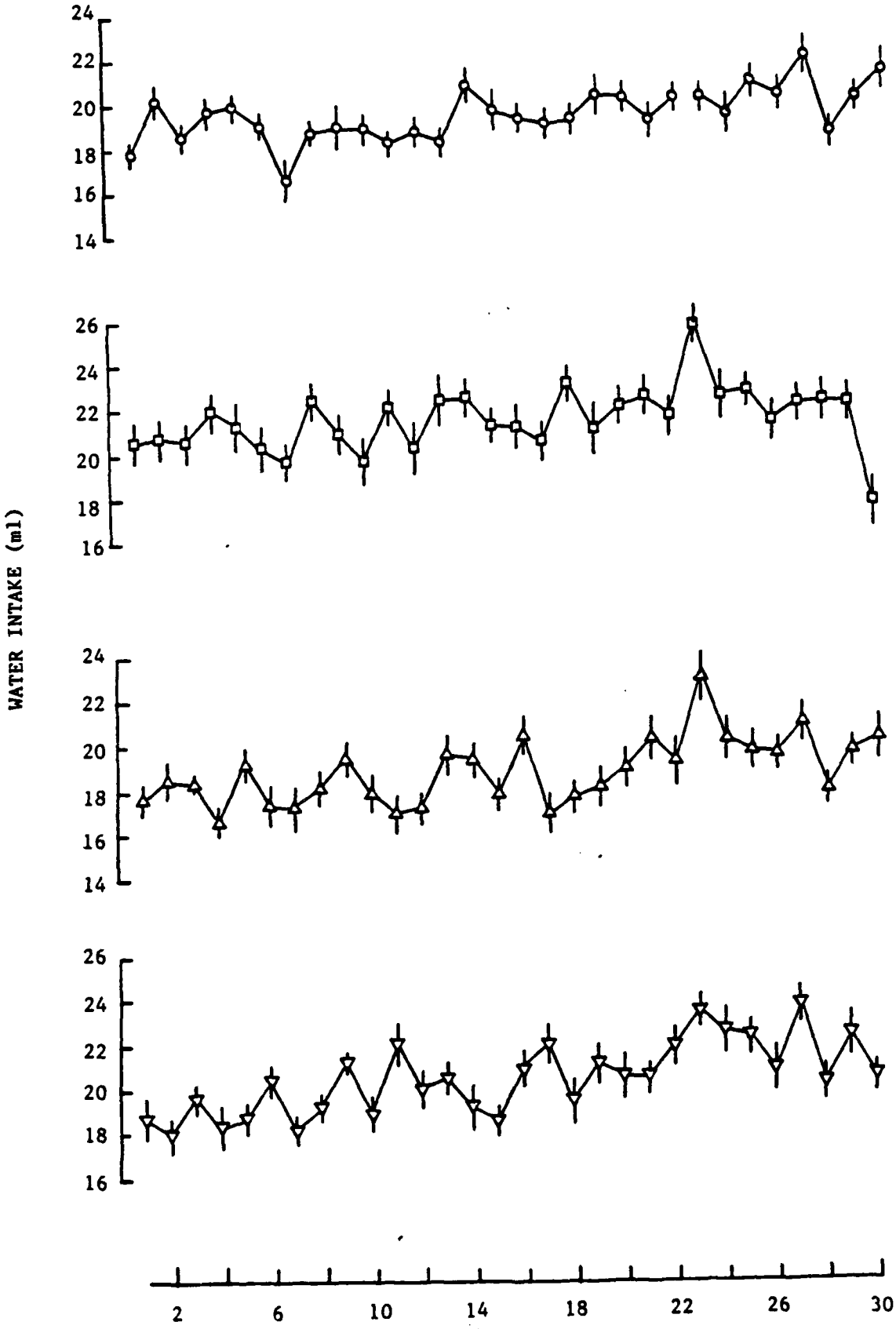

Figure 27. Subchronic effects of RDX on restricted water intake. Each symbol represents the mean (+SEM) effect of RDX (squares, 1 mg/kg; triangles, 3 mg/kg; inverted triangles, 10 mg/kg) or RDX vehicle (circles) (N=18 male rats/group) on daily water intake (30 min availability/day), across the 30 days of the subchronic dosing regimen.

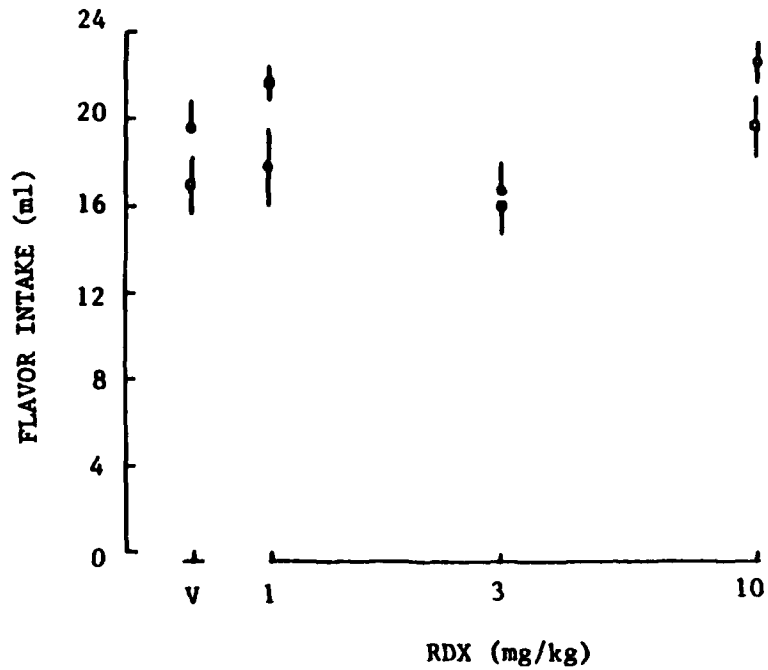

Figure 28. Subchronic effects of RDX on restricted saccharin intake. Each symbol represents the mean (±SEM) effect of RDX or RDX vehicle (N=18 male rats/group) on saccharin intake (available for 30 min) on the day after the last of the 30-day treatment regimen. Circles represent rats designated to receive saline and squares represent rats designated to receive lithium chloride.

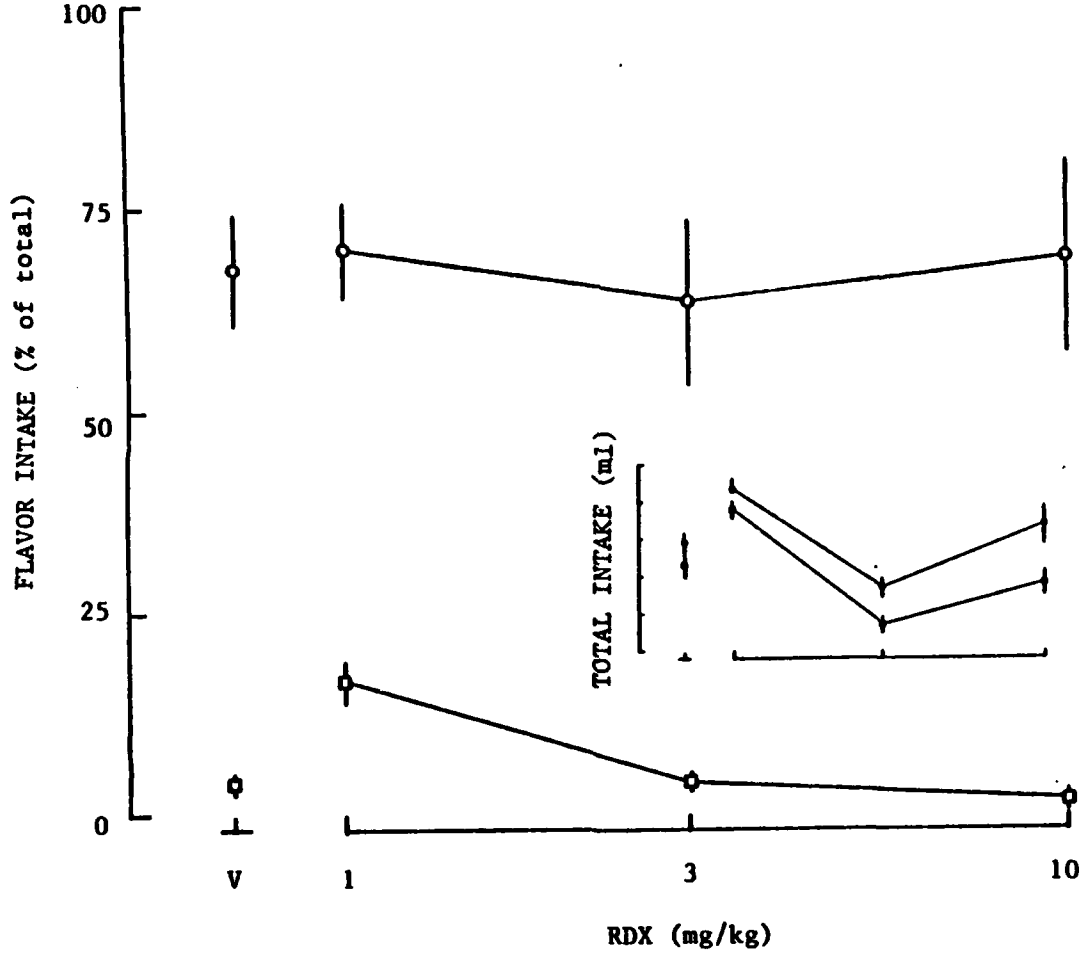

Figure 29. Subchronic effects of RDX on lithium chloride-induced flavor-aversion conditioning in male rats. One day after the last of a 30-day daily dosing regimen all rats were first given access to saccharin solution (see Figure 27) and then injected with either lithium chloride (0.9 mEq/kg) or the isotonic saline vehicle. Choice testing occurred 3 days later. Each symbol represents the mean (±SEM) saccharin intake, expressed as a percentage of total intake, and plotted as a function of subchronic dosing treatment (N=9/group). Insert shows treatment effects on total intake.

treated rats. The conditioned flavor aversions obtained in rats that had received 1 mg/kg RDX subchronically were somewhat less than those obtained in the other treatment groups. An analysis of variance revealed, however, that there was a statistically reliable effect of lithium chloride administration ($F = 177.1$, $df = 1,64$, $P<0.001$), regardless of RDX-dosing history, and no lithium-by-RDX interaction. The insert in Figure 29 shows that subchronic RDX effects on total fluid intake during the choice session were not systematically related to RDX dosage.

4. Schedule-controlled behavior. Rats were first trained to respond under a VR-50 schedule of milk delivery. Once performances had stabilized, behavioral testing was suspended and separate groups of rats were treated daily with either a dosage of RDX or RDX vehicle. All rats were retested for 5-day periods beginning on day 13 of dosing and again on day 31 (i.e., one day after discontinuation of daily dosing). The results of this experiment are shown in Figure 30. Baseline rates were comparable among the different designated treatment groups (left panel). No differences in performance were apparent between RDX-treated and vehicle-treated rats during the retest period beginning on day 13 (center panel). Comparable data for the retest period beginning on day 31 are shown in the right panel. Disruptions in performance were initially seen in vehicle-treated rats during this retest period. No disruptions in performance were seen in RDX-treated rats regardless of the dosage that had been administered daily. An analysis of variance revealed that there was a statistically significant effect of treatment on days 31 through 35 of testing ($F = 16.02$, $df = 3,140$, $P<0.001$), but no effect of days or days-by-treatment interaction.

5. Acoustic startle response. Rats were treated daily with either a dosage of RDX or RDX vehicle. Separate groups of rats were then tested

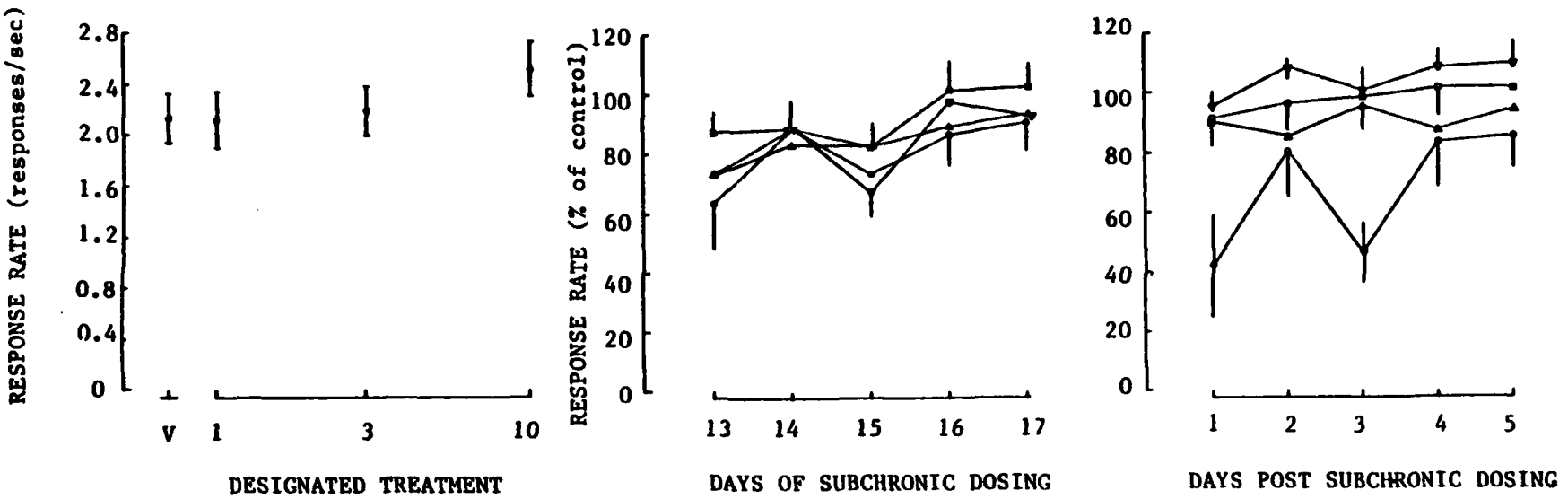

Figure 30. Subchronic effects of RDX on schedule-controlled behavior. Left panel shows mean (±SEM) baseline response rates for each of the designated treatment groups (N=8 male rats/group). Center and right panels show the mean (±SEM) effects of RDX (squares, 1 mg/kg; triangles, 3 mg/kg; inverted triangles, 10 mg/kg) or RDX vehicle (circles expressed as a percentage of baseline rates, obtained during and after, respectively, the subchronic dosing regimen.

in acoustic startle response chambers on day 16 or on day 31 of the dosing regimen. These data are shown in Figures 31 and 32. On day 16 (i.e., one day after the 15th daily treatment), no differences in either the amplitude or the latency of the acoustic startle response could be detected between RDX-treated and vehicle-treated rats (Figure 31). Similarly, no differences were observed on day 31 in the acoustic startle response of treated and control rats (Figure 32).

6. Analytical. RDX was not detectable in tissues taken from rats (N=10/group) receiving 15 daily treatments of 1 or 3 mg/kg. In rats receiving 10 mg/kg, RDX was measurable in the plasma of one rat (0.72 μg/ml) and in the brains of two rats (2.15 and 0.18 μg/gm). However, group-mean concentrations of RDX in both brain (0.23 μg/gm) and plasma (0.07 μg/ml) were very low at this time. After 30 daily exposures, RDX was not detected in the plasma in any of the dosage groups (N=10/group), and was measurable in the brains of only two rats (1.42 and 1.27 μg/gm) that had received 10 mg/kg. Group-mean RDX concentration in the brains of rats that had received 10 mg/kg was very low (0.27 μg/gm).

IV. DISCUSSION AND CONCLUSIONS

Acute administration of RDX to rats produced a wide range of behavioral effects. RDX substantially decreased the overall level of motor activity measured in the figure-eight maze and also altered its temporal and spatial distributions. RDX decreased hindlimb splay in the landing footspread test, but not in a dosage-related fashion. RDX also produced decreases in the amplitude, and increases in the latency, of the acoustic startle response. Moreover, RDX produced substantial decreases in the response rates of rats performing under VI-90-sec and VR-50 schedules of reinforcement. These behavioral effects were obtained in the absence of gross clinical signs

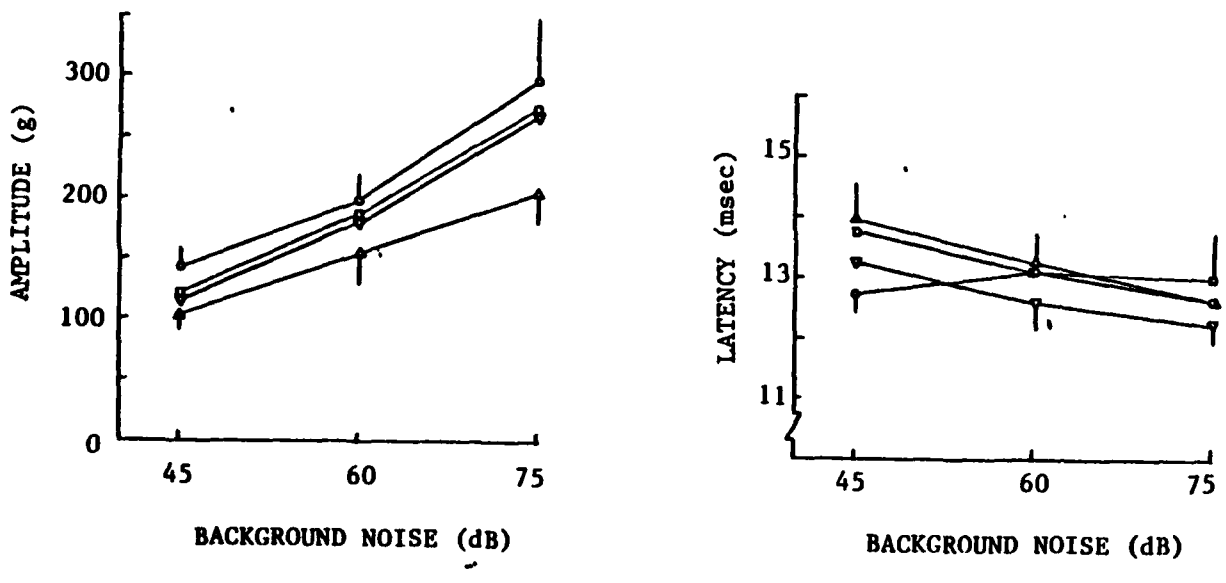

Figure 31. Subchronic effects of RDX on the acoustic startle response in male rats. Left panel shows mean (±SEM) amplitude and right panel shows mean (±SEM) latency of the startle response, plotted as a function of background noise intensity, for RDX-treated (squares, 1 mg/kg; triangles, 3 mg/kg; inverted triangles, 10 mg/kg) and vehicletreated (circles) rats (N=10/group) determined after the 15th daily treatment.

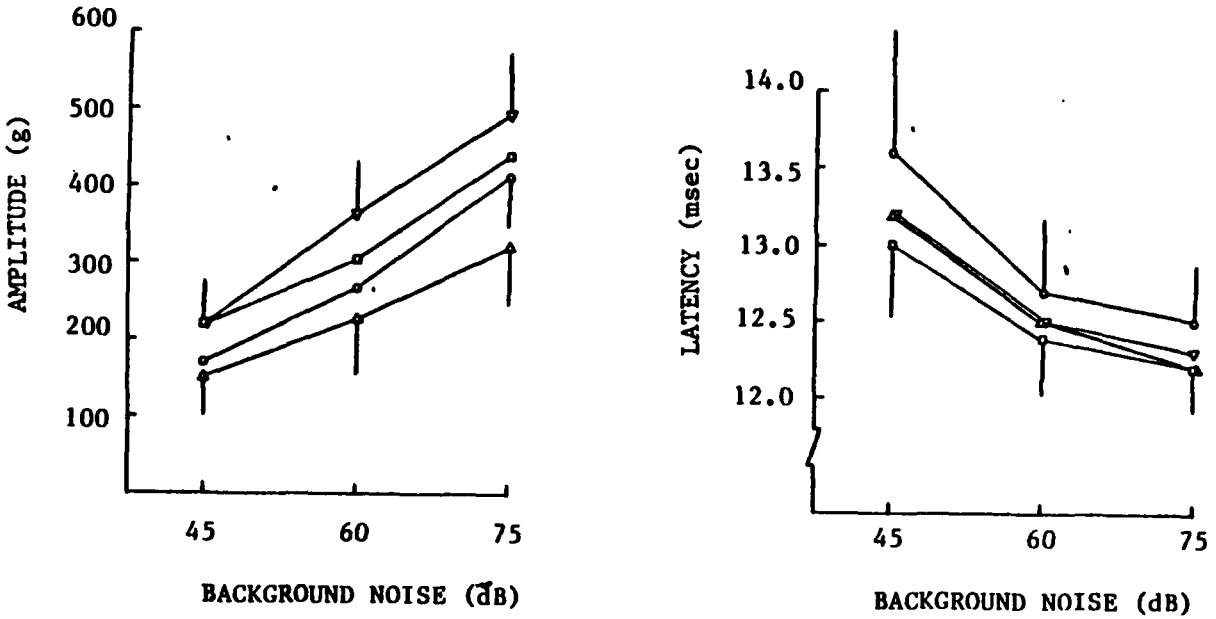

Figure 32. Subchronic effects of RDX on the acoustic startle response in male rats. Left panel shows mean (±SEM) amplitude and right panel shows mean (±SEM) latency of the startle response, plotted as a function of background noise intensity, for RDX-treated (squares, 1 mg/kg; triangles, 3 mg/kg; inverted triangles, 10 mg/kg) and vehicle-treated (circles) rats (N=10/group) determined after the 30th daily treatment.

of intoxication. Furthermore, carry-over effects of acute administration were apparent in many instances (e.g., for motor activity, total fluid intake in the flavor-aversion conditioning paradigm, and schedule-controlled performance). Taken together, the results indicate that acute RDX administration produces a wide range of sensory, motor and cognitive effects in rats. LD50 values reported in the literature range from 100 mg/kg (12) to 500 mg/kg (Sklyanskaza and Pozhariskii, 1944, as reported in 4). LD50 values are heavily influenced by particle size of as well as the vehicle used to deliver RDX (12). In the present series of experiments, acute dosages ranged from 12.5 to 50 mg/kg. Although direct comparisons are difficult, it nevertheless appears that acutely administered RDX produces prominent behavioral effects at dosages that are 10% or less of the acute LD50.

RDX concentrations in plasma and in brain were a direct function of the acute dosage administered. Furthermore, a relatively large dosage of RDX was rapidly taken up by plasma and brain and retained in these tissues for at least 24 hours. When the time course for retention was determined over longer periods of time, substantial levels of RDX were found up to 48 hours after dosing but were undetectable at 72 and 96 hours after dosing. The plasma data agree fairly well with those of Schneider et al. (12). A non-linear relationship between log-transformed RDX concentration in both plasma and brain and time since dosing would indicate that RDX is not distributed into a single-compartment system and that elimination cannot be described by first-order kinetics. The prolonged elimination of RDX, together with evidence of carry-over effects on behavior the day after acute exposures to RDX, suggested that cumulative toxicity was likely to be obtained with subchronic exposures to RDX. The dosages used

in the subchronic experiments were therefore selected to produce a range of behavioral effects, from cumulative effects to no effect.

In contrast to predictions, very little evidence of behavioral toxicity was obtained when RDX was administered daily for up to 30 days. In addition, RDX levels in plasma and in brain were generally undetectable approximately 24 hours after either the 15th or the 30th daily treatment with RDX. The general absence of behavioral effects in the subchronic experiments could be due to several different factors. It is possible, of course, that tolerance could have developed to the behaviorally disruptive effects of RDX on subchronic administration. This possibility could be evaluated directly by sequentially establishing acute dose-response functions, determining the effect of acute behaviorally disruptive dosages when given daily, and then redetermining the acute dose-response function for RDX following a history of daily RDX dosing. If tolerance did develop, variations on the experimental design (e.g., post-test daily dosing) could be incorporated to delineate the relative importance of daily exposures to RDX _per se_ and daily RDX exposures while testing was taking place.

It is also conceivable that the relative lack of effectiveness of RDX in the subchronic experiments could have been due to either the dosages or the times selected for testing the animals after dosing. Dosage selection was based on (1) the large effects produced by acute exposures to relatively small dosages of RDX and (2) the finding that RDX persisted in plasma and brain for a relatively long time following a large dosage. In addition, on the basis of extrapolating to smaller dosages from the acute dosage-concentration experiment, the largest dosage used in the subchronic experiment (10 mg/kg) should have resulted in significant RDX concentrations in plasma and in brain. The relative lack of effect of RDX,

along with the absence of RDX in plasma and brain after daily exposures to dosages which would otherwise result in significant RDX concentrations after acute exposure, suggests that RDX follows rates of absorption and elimination which depend on the dosage administered. Numerous other compounds display kinetics that are determined by the dosage administered. For example, Piper et al. (9) showed that plasma levels of 2,4,5-T decreased with a half-life of 25.2 hours when rats were intubated with 200 mg/kg. When, however, rats were intubated with 50 mg/kg, the half-life for plasma was considerably shorter (4.2 hours). Levy (7) has reviewed a number of other compounds that display dose-dependent pharmacokinetics. Additional experiments are clearly required to determine whether the kinetics of RDX, and its metabolites, are indeed dose dependent. Possible underlying mechanisms might include dose-dependent effects on: gastrointestinal absorption; distribution; metabolism and/or elimination (see 7).

Time of testing in the subchronic experiment was selected so as to minimize the contribution of any one daily RDX exposure to the observed behavioral effects. The relative lack of effect of RDX in the subchronic experiments, however, suggests that further research should include additional times of testing relative to dosing as well as an expanded range of dosages.

The signs of toxicity in humans exposed to RDX include neuromuscular changes (especially altered reflexes), gastrointestinal involvement (including nausea), as well as cognitive deficits and seizures. Broadly speaking, the results of the acute exposure experiments are consistent with these signs. Specifically, RDX produced alterations in motor activity, and in those reflexes involved in landing footspread and the acoustic startle response. The remarkable potency of RDX in inducing conditioned flavor aversions may in fact be reflective of the gastrointestinal complaints

expressed by humans. In addition, RDX-induced disruptions of schedule-controlled performance may reflect altered cognitive function, although the precise nature of the deficit requires further investigation. Moreover, Burdette and Dyer (1) recently found in rats that acute RDX exposures produced both spontaneous seizures and enhanced susceptibility to audiogenic and chemically-induced seizures. Together with the present results, these findings suggest that key components of an animal model of human acute RDX intoxication have been tentatively identified.

V. RECOMMENDATIONS

The results of these experiments indicate that RDX produces a multitude of behavioral effects on acute administration. Following large dosages these effects are likely to persist for days after exposure. In contrast, very little evidence of behavioral toxicity was obtained following subchronic exposures to smaller dosages. Future research should be directed toward defining the conditions under which subchronic RDX produces behavioral toxicity by using a wide variety of dosing protocols (including varying dosages, chronicities and times of testing relative to dosing). In addition, future studies should be directed toward determining the metabolism and toxicokinetics of a range of RDX dosages following both acute and subchronic dosing protocols, and using a variety of tissues taken from specific regions of the central and peripheral nervous system.

VI. REFERENCES

1. Burdette, L.J. and R.S. Dyer. RDX exposure sensitizes rats to audiogenic seizures. The Toxicologist 5: 25, 1985.

2. Edwards, P.M. and V.H. Parker. A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats. Toxicol. Appl. Pharmacol. 40: 589-591, 1977.

3. Fleshler, M. and H.S. Hoffman. A progression for generating variable interval schedules. J. Exper. Anal. Behav. 5: 529-530, 1962.

4. Kaplan, A.S., C.F. Berghout and A. Peczenik. Human intoxication from RDX. Arch. Environ. Hlth. 10: 877-883, 1965.

5. Knepshield, J.H. and W.J. Stone. Toxic effects following ingestion of C-4 plastic explosive. In: Drug abuse: Current concepts and research. W. Keup (Ed.), Springfield, IL: Charles C. Thomas, publisher, 1972.

6. Levine, B.S., E.M. Faredi, D.E. Gordon, J.M. Burns and P.M. Lish. Thirteen week toxicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Fisher 344 rats. Toxicol. Lett. 8: 241-245, 1981.

7. Levy, G. Dose dependent effects in pharmacokinetics. In: Importance of Fundamental Principles in Drug Evaluation. Ed. by D.H. Tedeschi and R.S. Tedeschi, New York, Raven Press, 1968.

8. MacPhail, R.C. Studies on the flavor aversions induced by trialkyltin compounds. Neurobehav. Toxicol. Teratol. 4: 225-230, 1982.

9. Piper, W.N., J.Q. Rose, M.L. Levy and P.J. Gehring. The fate of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) following oral administration to rats and dogs. Toxicol. Appl. Pharmacol. 26: 339-351, 1973.

10. Reiter, L.W. Chemical exposures and animal activity: Utility of the figure-eight maze. In: Developments in the science and practice of toxicology. A.W. Hayes, R.C. Schnell and T.S. Miya (Eds.), New York, Elsevier Science Publishers B.V., 1983.

11. Ruppert, P.H., K.F. Dean and L.W. Reiter. Trimethyltin disrupts acoustic startle responding in adult rats. Toxicol Lett. 22: 33-38, 1984.

12. Schneider, N.R., S.L. Bradley and M.E. Anderson. Toxicology of cyclotrimethylenetrinitramine: Distribution and metabolism in the rat and the miniature swine. Toxicol. Appl. Pharmacol. 39: 531-541, 1977.

13. Schneider, N.R., S.L. Bradley and M.E. Anderson. The distribution and metabolism of cyclotrimethylenetrinitramine (RDX) in the rat after subchronic administration. Toxicol. Appl. Pharmacol. 46: 163-171, 1978.

14. von Oettingen, W.F., D.D. Donahue, H. Yagoda, A.R. Monaco and M.R. Harris. Toxicity and potential dangers of cyclotrimethylenetrinitramine (RDX). J. Ind. Hyg. Toxicol. 31: 21-31, 1949.

APPENDICES

1. Efficiency and Sensitivity of Extraction into Ethyl Acetate.

The efficiency of RDX extraction into ethyl acetate from brain homogenate, plasma and water was determined. The concentration of RDX in 0.5 ml extractions after spiking brain homogenate, plasma and water with a given amount of RDX was compared to the RDX concentration resulting from the addition of the same amount of RDX directly to 0.5 ml of ethyl acetate. The efficiency of extraction of RDX from brain homogenate, plasma and water was 95%, 91% and 100% respectively. The efficiency of the extraction was also assessed by repeated extractions of brain homogenate and plasma samples. Trace amounts of RDX could be detected in the second extractions, probably due only to the incomplete removal of the original ethyl acetate extraction.

The sensitivity of the analysis was calculated as the smallest concentration of RDX in brain homogenate and plasma which could be detected. The limitations of sensitivity using the present conditions of analysis are the degree of absorption of ethyl acetate at 254 nm and similarity of retention times of peaks resulting from ethyl acetate and RDX. The sensitivity of RDX analysis is approximately 200 ng/ml of brain homogenate and 500 ng/ml of plasma using the procedure described above. The sensitivity can be improved by evaporating the ethyl acetate extraction to dryness followed by dissolving the residue in methanol.

2. Solubility of RDX in corn oil.

In an attempt to prepare a dosing solution of RDX in corn oil, 0.5 ml of a solution of 50 mg RDX/ml acetone was added to a calibrated 2 ml reaction tube followed by 0.5 ml of corn oil. This solution was mixed and placed under vacuum for approximately 16 hrs. This resulted in a mixture of apparently crystalized RDX in 0.5 ml of corn oil.

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